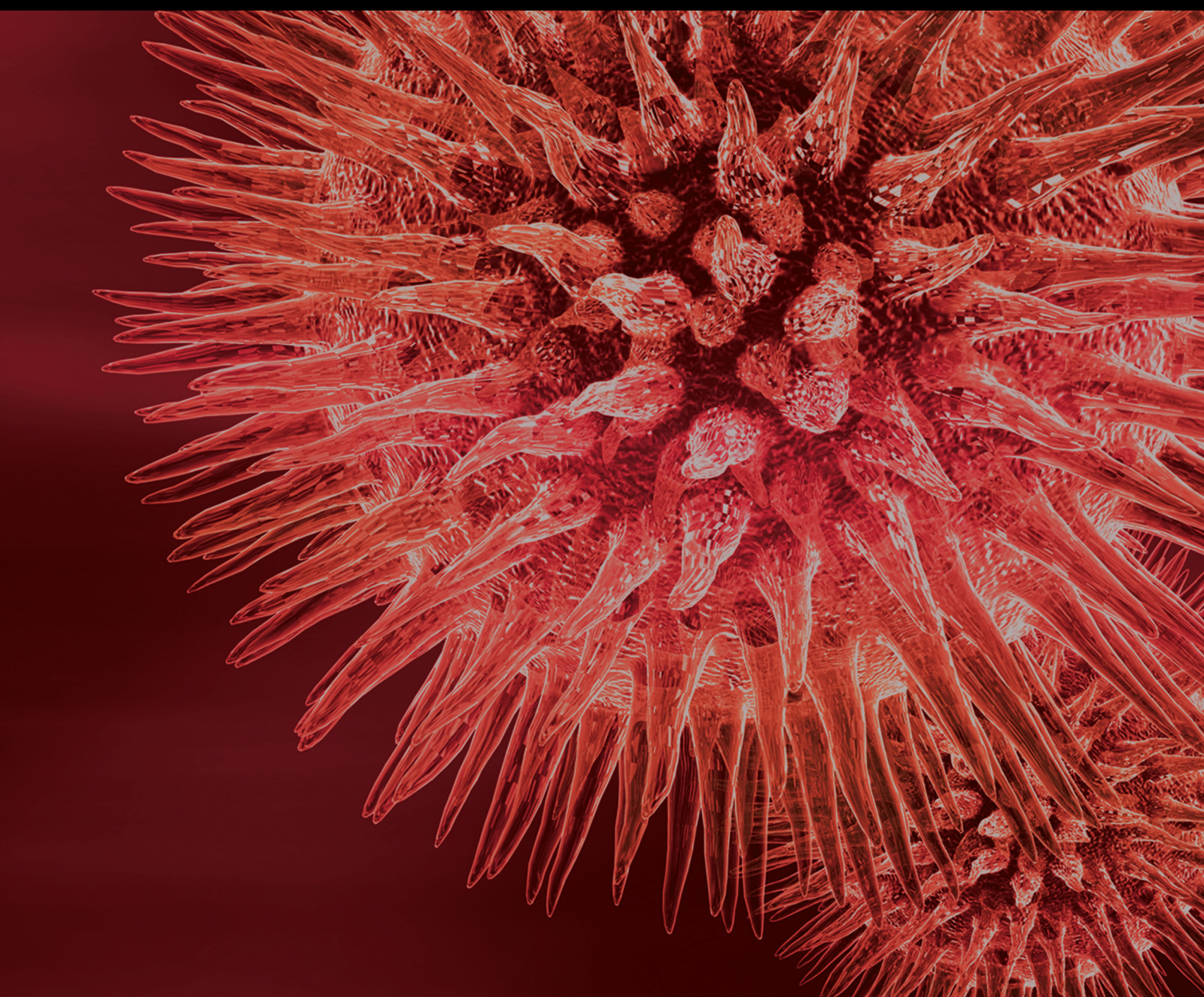
BioMed Research International

Personalized Approach to Severe Asthma

Special Issue Editor in Chief: Enrico Heffler
Guest Editors: Giorgio W. Canonica, Zuzana Diamant, Joao Fonseca, and Andrei Malinovschi



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Contents

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Editorial

Personalized Approach to Severe Asthma

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Approximately 5-10% of asthmatics are barely controlled or clinically and/or functionally uncontrolled despite a high dose of inhaled corticosteroids (ICS) plus another controller agent (e.g., long-acting beta2-agonists, LABA; leukotriene-receptor antagonists, LTRA; long-acting muscarinic agents, LAMA) or maintenance oral corticosteroid therapy. These patients are defined as affected by "severe asthma" according to the most recent recommendations of the European Respiratory Society (ERS) and the American Thoracic Society [1]. The diagnosis of severe asthma is made after having ruled out or having treated clinical conditions that may mimic asthmatic symptoms (e.g., extra-thoracic hyperresponsiveness syndromes, vocal cord dysfunction), comorbidities that may worsen disease control (e.g., allergic or nonallergic rhinitis, chronic rhinosinusitis with or without nasal polyps, bronchiectasis, and gastroesophageal reflux), possible incorrect inhaler techniques, and/or poor treatment adherence. During the past decade, advanced research brought insight into the heterogeneous mechanisms of severe asthma and helped to reveal several potential therapeutic targets [2].

Following the introduction of the first available biologic agents in clinical practice, the way of diagnosing and managing the majority of patients with severe asthma dramatically changed from a "one-size-fits-all" approach to precision medicine [3]. Presently, we are experiencing a new era in the management of severe asthmatic patients, as subjects are clinically characterized in phenotypes [4] or in treatable traits [5] in order to personalize their disease-management.

In this special issue, the latest knowledge and novel findings in severe asthma pathogenesis, pheno/endotyping and management with a particular focus on personalized and precision medicine approaches, have been addressed.

The classification of patients according to their phenotypes and/or endotypes [4] is strictly dependent on the identification of reliable biomarkers, ideally noninvasive and available for point-of-care [6]. The article by E. Mortaz et al. elegantly summarizes a plethora of possible new biomarkers from tissue-derived exosomes. These small membrane-enclosed vesicles contain mRNA and miRNA, lipids, and a vast array of different proteins depending on their cell of origin. Furthermore, exosomes may also be potentially used for developing novel therapeutic strategies. C. Galeone et al. pointed their attention on how the new field of "omics" sciences (including proteomics, metabolomics, transcriptomics, and genomics) may provide new biomarkers, novel targets for diagnostic tests, and pharmacological treatments.

This complex scenario of new technologies and biomarkers, applied to the process of identification of specific severe asthma phenotypes and endotypes, is part of the precision medicine approach to asthma. The direct consequence of a better characterization of patients under the immunological point of view is the possibility to treat them with novel

biologic agents, acting directly towards those immunological mechanisms that are involved in every single endotype of severe asthma [7]. The first available biologic agent for severe asthma was omalizumab, a fully humanized anti-IgE monoclonal antibody. Its clinical efficacy and effectiveness in severe allergic patients have been proved extensively. There are some recent studies also suggesting effectiveness in nonallergic severe asthmatics. C. C. Loureiro et al. reviewed the current evidence on both of these possible uses of omalizumab in this present Special Issue. In the past few years, novel therapeutic targets have been addressed by recently approved biologic agents: mainly, anti-IL5 strategies are currently worldwide used for severe eosinophilic asthma [8]. D. Bagnasco et al. overviewed the possible molecular targets and related biologic drugs, blocking the IL5-mediated eosinophilic inflammation in severe asthma. C. Pelaia et al. dedicated their review article to the specific mechanism and clinical effects of benralizumab, an afucosylated monoclonal antibody towards the IL5-receptor, a newly approved drug for treatment of severe asthma in 2018. This drug has a remarkable affinity for the FcγRIIIa receptor of NK cells that gives to the drug the ability to induce the apoptotic mechanism named antibody-dependent cell-mediated cytotoxicity (ADCC). L. Brussino et al. critically revised the published literature on anti-IL5 treatment in asthma and highlighted the still present unmet needs, critical points, and open questions on efficacy and real-life effectiveness of this category of drugs in severe asthmatics.

Beyond the use of biologic agents, among the approved drugs for severe asthma, the inhaled LAMA tiotropium may play a role, both before starting any biologic treatment and for those patients not meeting the indication for any of the currently available biologics. E. Hamelmann et al. reviewed the evidence of the use of inhaled tiotropium in severe asthmatics.

A critical point for all treatments for severe asthma is the *a priori* identification of responder patients: this depends on many variables (e.g., clinical, functional and immunological characteristics, associated comorbidities) that may not correspond precisely to the features of the extremely selected patients included into randomized-controlled trials. Therefore, real-life big-data on severe asthma are needed to improve the characterization of our patients and provide them with adequate and tailored treatment. A very effective approach to obtain real-life big-data is establishing national and international registries [9, 10], as they will include a large amount of information on "real" patients managed by physicians in their daily clinical activity. A. Sá-Sousa et al., in this special issue, described the protocol and the aims of such an initiative, the Portuguese Severe Asthma Registry.

In conclusion, this special issue updates and summarizes recent knowledge on many different aspects of severe asthma.

Conflicts of Interest

Prof. Giorgio Walter Canonica is a member of advisory boards, speaker, scientific meetings for GSK, Teva, Sanofi, Roche, Novartis, AstraZeneca.

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Research Article

The Portuguese Severe Asthma Registry: Development, Features, and Data Sharing Policies

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The Portuguese Severe Asthma Registry (*Registo de Asma Grave Portugal,* RAG) was developed by an open collaborative network of asthma specialists. RAG collects data from adults and pediatric severe asthma patients that despite treatment optimization and adequate management of comorbidities require step 4/5 treatment according to GINA recommendations. In this paper, we describe the development and implementation of RAG, its features, and data sharing policies. The contents and structure of RAG were defined in a multistep consensus process. A pilot version was pretested and iteratively improved. The selection of data elements for RAG considered other severe asthma registries, aiming at characterizing the patient's clinical status whilst avoiding overloading the standard workflow of the clinical appointment. Features of RAG include automatic assessment of eligibility, easy data input, and exportable data in natural language that can be pasted directly in patients' electronic health record and security features to enable data sharing (among researchers and with other international databases) without compromising patients' confidentiality. RAG is a national web-based disease registry of severe asthma patients, available at *asmagrave.pt*. It allows prospective clinical data collection, promotes standardized care and collaborative clinical research, and may contribute to inform evidence-based healthcare policies for severe asthma.

1. Introduction

Severe asthma has been defined as asthma which requires treatment with high dose inhaled corticosteroids plus a second controller (and/or systemic corticosteroids), to prevent it from becoming "uncontrolled" or asthma which remains "uncontrolled" despite this therapy [1].

To improve care, a better understanding of the etiology, burden and management patterns of severe asthma is needed. The management of severe asthma is challenging and involves treatment of comorbidities, medication adherence, allergens exposure avoidance, among others. One of the greatest difficulties is the choice of the optimal treatment for each given patient, although algorithms for treatment decisions have been suggested [2, 3]. Monoclonal antibodies targeting immunoglobulin-E (IgE) and interleukin-5 are currently available and new biologics are under development. However, it is not easy to choose between the biologics to be the first-choice treatment, and head-to-head comparison studies between them do not exist [4]. A trial involving the direct comparison of two or more treatments is a pressing needed, but it may never be carried out [4]. Hence, clinical observational studies of real-world large patient populations should contribute to the knowledge on how to select the best biologic treatment for an individual patient.

Disease registries are recognized as powerful tools to improve disease-related knowledge. They consist of organized systems that use observational study methods to collect uniform data aiming at evaluating specific outcomes for a heterogeneous population defined by a particular disease [5]. This type of study design enables the assessment of the effect of different therapies in the context of a single disease. Severe asthma registries are being created throughout Europe including in the United Kingdom (UK), Belgium, Germany, Austria, Netherlands, Italy, and Spain (Table 1). However, research aiming at reducing the disease-related burden requires prospective long-lasting studies and the coordination of a wide range of expertise, often only available

Table 1: European Registries of Severe Asthma, a noncomprehensive review.

Registry name	Country	Year of release	Promoting Society	Website	Patients included	No. of centers	Sources / published studies
United Kingdom Severe Asthma Registry	United Kingdom	2006	British Thoracic Society	https://www.brit-thoracic.org.uk/standards-of-care/lung-disease-registries/	>500	8	[13–22]
Belgian Severe Asthma Registry	Belgium	2008	Belgische Vereniging voor Pneumologie / Société Belge de Pneumologie	http://www1.citobi.be/SAR/Welcome_en.act	>350	9	[23, 24]
Register Schweres Asthma	Germany	2011	German Asthma Net e.V.	http://www.german-asthma-net.de	>100	17	[25, 26]
Banco de Datos de Asma	Spain	<2012	Sociedad Española de Neumologia y CirurgiaToracica	https://www.separ.es/?q=node/71	>290	30	[27, 28]
Austrian Severe Asthma Net	Austria	2012	Austrian Severe Asthma Net (ASA-Net)	http://www.asa-net.at/register/	>80	16	[29]
Severe/Uncontrolled Asthma Registry	Italy	2014	Italian Severe Asthma Network (SANI).	http://www.sani-asma.org	>400	63	[30, 31]
Registry of Adult Patients with Severe asthma for Optimal Disease management	Netherlands	2016	Academisch Medisch Centrum (Prof. dr. E.H.D. Bel)	https://www.zonmw.nl/nl/over-zonmw/innovatie-in-de-zorg/programmas/project-detail/goed-gebruik-geneesmiddelen/registry-of-adult-patients-with-severe-asthma-for-optimal-disease-managementrapsodi/verslagen/	>20	3	[32]
Registo de Asma Grave Portugal	Portugal	2018	Rede de Especialistas em Asma Grave	https://www.asmagrave.pt/	Release planned for 2nd trimester of 2018	31	-

at an international or even global level [6]. With the goal of establishing a global collaborative initiative, the International Severe Asthma Registry was created and the enrollment of 10 national registries is expected by December 2018[7]. The European Respiratory Society (ERS) Research Agency promotes collaborative Europe-wide research based on data collected from disease registries [8]. Its actions include the development of Standard Operational Procedures and guidelines, consent forms to collect and handle data in compliance with the EU legal and regulatory framework, and establishing a central point to access datasets from multiple projects. In 2016 the collaboration Severe Heterogeneous Asthma Research collaboration, Patient-centered (SHARP) was accepted as an ERS Clinical Research Collaborations [9]. Taking this into consideration, new registries should be designed to enable sharing information and coordination among databases (e.g., federated databases).

Asthma affects 6.8% of the Portuguese population [10]. Using the data from the Portuguese National Asthma Survey we estimate 7.4% of patients were on step 4 or 5 treatment as defined by Global Initiative for Asthma (unpublished data). Even though severe asthma patients represent only a small proportion of those with asthma, they account for a large proportion of asthma-related morbidity and health care expenditures [11].

REAG, *Rede de Especialistas em Asma Grave*, is an open collaborative network of asthma specialists (allergists, pediatricians, and pulmonologists) who manage severe asthma patients in Portuguese hospitals. The foundational principle of REAG is the informal peer collaboration among colleagues with different medical specialties and backgrounds, maintaining an unhierarchical organization and consensual decision processes to improve sharing of medical experience, data, and knowledge. Since 2011, this network of experts has been working towards a better care of severe asthma patients by (1) promoting a better coordination between medical specialties for early diagnosis and referral of severe asthma patients; (2) describing and implementing harmonized procedures to adopt in severe asthma healthcare; and (3) improving scientific knowledge on severe asthma in Portugal. In 2015, REAG published a real-life prospective study on Portuguese patients with severe persistent allergic asthma, treated with omalizumab [12]. This was the first-time specialists from different Portuguese centers who made an effort to harmonize the registration procedures for severe asthma. From this initial study, the necessity for a computerized disease registry became even more evident.

The purpose of the Portuguese Severe Asthma Registry (*Registo de Asma Grave Portugal (RAG)*) is to gather evidence on severe asthma in Portugal contributing to eliminate the information gaps and support the adoption of evidence-based health care policies. Specifically, the registry aims at

(1) improving the healthcare delivery of severe asthma by identifying the best diagnosis and treatment practices and by standardizing disease management processes and clinical records;

(2) supporting collaborative research projects by promoting the cooperation between centers and assist with the implementation of research projects.

In this paper, we describe the development and implementation of RAG, its features, and data sharing policies.

2. Material and Methods

RAG results from the collaboration between different stakeholders: the medical experts from REAG, the investigators from CINTESIS (Center for Health Technology and Services Research), and the software development company VirtualCare.

The development and implementation processes of RAG are summarized in Figure 1.

2.1. Definition of Contents. The criteria for patient inclusion in RAG, the domains, and data elements to be registered were defined by a multistep consensus method.

The patients' inclusion criteria were based on the definition of Severe Asthma by GINA [1]: (1) patient with treatment on step 4 or 5 according to GINA recommendations; and (2) verified optimization of treatment adherence and comorbidities management. An additional inclusion criterion was (3) the patient's signed consent to have his/her data included in the registry.

During a meeting (April 2016), the domains and data elements were enumerated, based on the medical expertise of the network and taking into consideration the variables existing in three existing European Registries: the Belgian, the German, and the UK Severe Asthma Registries. Both data elements to be included in the initial patient registry and relevant follow-up information were identified. Different data entry methods were considered to reduce the burden of response.

An online questionnaire sent to 79 medical specialists from REAG was used to explore the importance of each data element and adequacy of data entry method. A total of 34 participants (43%) completed the questionnaire. For each domain, data elements and methods for data entry were chosen when gathering at least 80% of the votes. Comments and suggestions regarding additional variables or different data entry methods were also considered. The results of the questionnaire were presented in a meeting (March 2017) and disagreements were solved by consensus.

2.2. Features. Database specifications concerning data definitions and parameters and data validation rules were determined. To assist confirmation of the first criterion and support decision-making, an algorithm to automatically determine the step of treatment based on currently used asthma medication was created.

The following additional features were implemented:

(i) Support on data entry by automatic validation of the inserted data and error messages

(ii) Creation of automatic reports, based on the information stored, to be integrated into the institutional electronic health record (the data recorded are exportable in natural language by generating a text that mimics clinical notes)

(iii) Graphic display of aggregated data on patients' inclusion by healthcare center

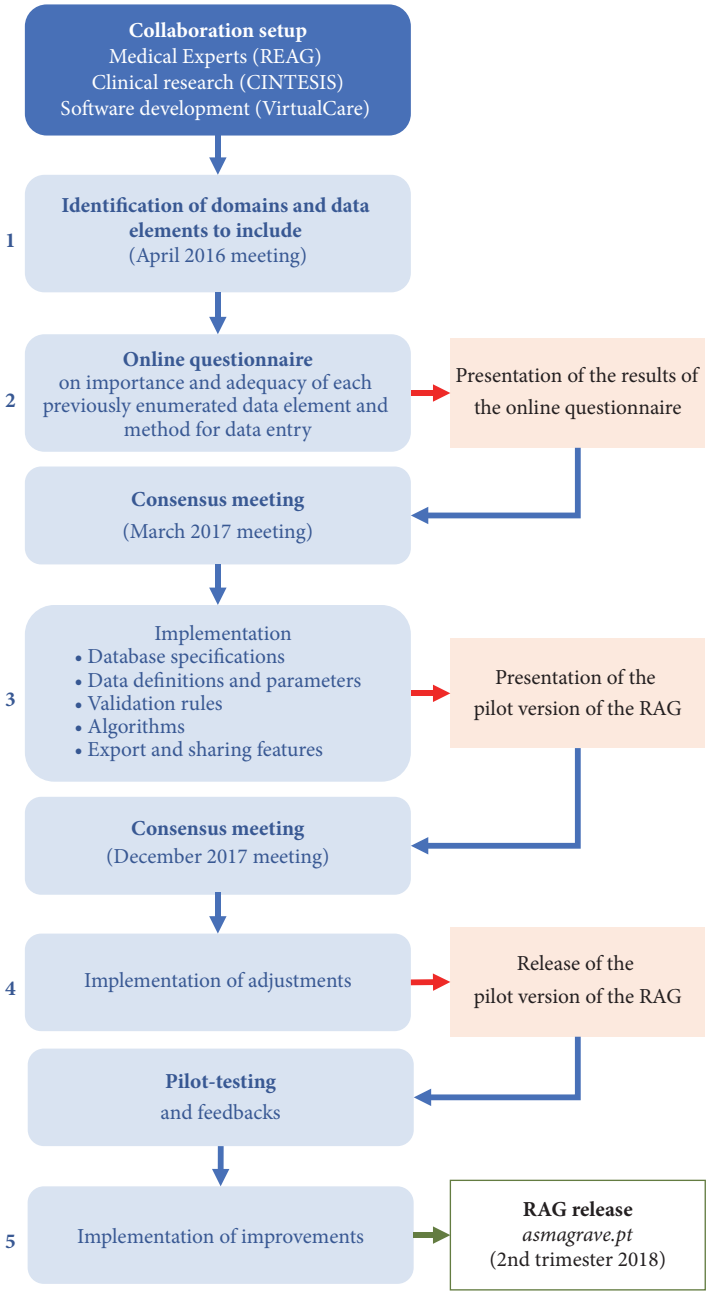

Figure 1: Development and implementation process of RAG.

(iv) Display for each physician a list of their patients and date of the last medical appointment

(v) At follow-up visit, automatic display of the information inserted in the last appointment for specified measurements

(vi) Export features for potential data exchange with international severe asthma databases and the pharmacovigilance authorities

(vii) Automatic emails with status report of each registration

2.3. Security and Data Sharing Policies. Security features compliant with the new European General Data Protection Regulation (GDPR) [37] and required procedures according to this legislation are being incorporated into the platform.

The registry was built on a framework residing in a server hosted by VirtualCare. This server was configured with a Secure Sockets Layer (SSL) certificate from Comodo Security Solutions, Inc., ensuring that all data transferred between the web server and browsers remain private and integral. The access to the database is restricted, requiring authentication (using health professional number and password) and all

accesses to the database are stored and traceable. All changes to the database are also stored; each change generates a new document; the old document becomes out of date allowing the tracking of changes (when, where, and by whom changes were made to the documents).

RAG does not record any identifiable personal data from patients (e.g., date of birth is replaced by the year of birth, no ID numbers are registered, and patients' names are pseudoanonymized so replaced with a code number) [38]. The patients' participation on RAG is free and voluntary, and patients may, in any moment and without penalty, withdraw the registry or verify and/or delete their data, by contacting the technical support. Patients are informed on the purposes of RAG, the data collected, and the implications of participating in this registry. The informed consent form is automatically generated at the time of inclusion. Only patients that agree, by a clear affirmative consent given by a written statement, to the storage, processing, and sharing of data belonging to him/her are included in RAG. The signed consent forms are upload into the application server file system, encrypted using phpseclib's library of PHP, which allows the usage of one of its encryption algorithms combined with a private key. When encrypted, the consent file cannot be read unless the file decryption is activated with the correct combination of algorithm and private key. The algorithm and private key are known only to VirtualCare.

An informed consent is also required by physicians who are registered in RAG since they provide identifiable personal data for that registration, namely, name, health professional number, and email address. At the time of registration, physicians must indicate their acceptance by ticking a box with a clear statement on the storage and processing of their personal data. The registration of each physician in RAG must be validated by at least one of five members of REAG, designated coordinators of RAG.

Data within RAG belongs primarily to each patient and then to the physician that inserted patients' data into the registry. Each physician is responsible for the management of the data that he/she inputted, belonging to his/her patients. Access to patients' data by their physicians is based on the Role Based Access Control (RBAC) model that associates privileges and permissions to the roles (e.g., professional categories). This model allows easier administration and independence in relation to the system users and permissions associated with its resources.

After authentication, each physician can access all the registrations inserted by himself/herself, both for clinical and research purposes. One local coordinator will be designated in each center. Each center coordinator has access, for pressing clinical purposes only, to all data inserted by the physicians in that center. If a patient changes the attending physician, the new physician, if interested in having access to the previously inserted data, must request authorization to the former physician, with patient's consent. Local and national coordinators and RAG technical support may assist this contact.

Data inserted by other physicians may be shared within REAG for research purposes, after authorization. For this, the physician proposing the data analysis must fill-in a form containing the aim and a brief description of the research project and the principal investigator or research group. When a request for abstracting data is filled, each physician with data matching the request is notified by email and has a period of 5 days to refuse the sharing of the data. In the case of shared information, the privacy of the individual is assured, as registry data cannot be individually identifiable.

2.4. Pilot-Test. After the implementation of the selected data elements, the supporting features, and validation rules, a beta version of RAG was presented during a REAG meeting (December 2017) and, after adjustments, it was pilot-tested for a month. The pilot version was tested by 22 REAG members and 85 specific feedback comments were provided by 8 testers. The first version of RAG became ready after improvements being made based on the pilot-test feedback.

3. Results

The Portuguese Severe Asthma Registry is a national web-based disease registry. The access is made from the website of REAG, *asmagrave.pt*, after authentication.

RAG gathers data of adults and children with severe asthma followed at specialized care centers which, after treatment optimization and adequate management of comorbidities, require step 4 or 5 of treatment according to GINA recommendations[1]. The implemented automatic algorithm determines the step of treatment for patients aged under 6, between 6 and 12 and over 12 years, based on asthma medication prescribed to the patient according to GINA recommendations (Figure 2.A). In any case, the physician makes the decision about the inclusion in the registry indicating the reason for inclusion (Figure 2.B). In fact, even if rarely used, some therapeutic combinations are not explicitly considered in any of the GINA 2018 treatment steps and in these cases, the algorithm cannot present a result. The algorithm will be updated in the future when these recommendations change.

The final data items of RAG are summarized in Table 2. RAG allows collecting data on different asthma medication, including Oral Corticosteroids (OCs), monoclonal antibodies, and even new therapies that may become available (Figure 3). Data considered as essential are compulsory, whereas desirable but not essential data may be skipped. The elements to be collected in the follow-up appointments were also defined as RAG was designed to collect data prospectively.

4. Discussion

The Portuguese Severe Asthma Registry is a national web-based disease registry of adult and pediatric severe asthma patients. It includes a comprehensive list of data elements defined by a multistep consensus process, supported by international definitions of severe asthma. The registry offers features to facilitate data entry and to support decision-making. The collected data belongs primarily to each patient and then to the physician who inserted patients' data into the registry and can be shared for research purposes after authorization. A thorough characterization of severe asthma

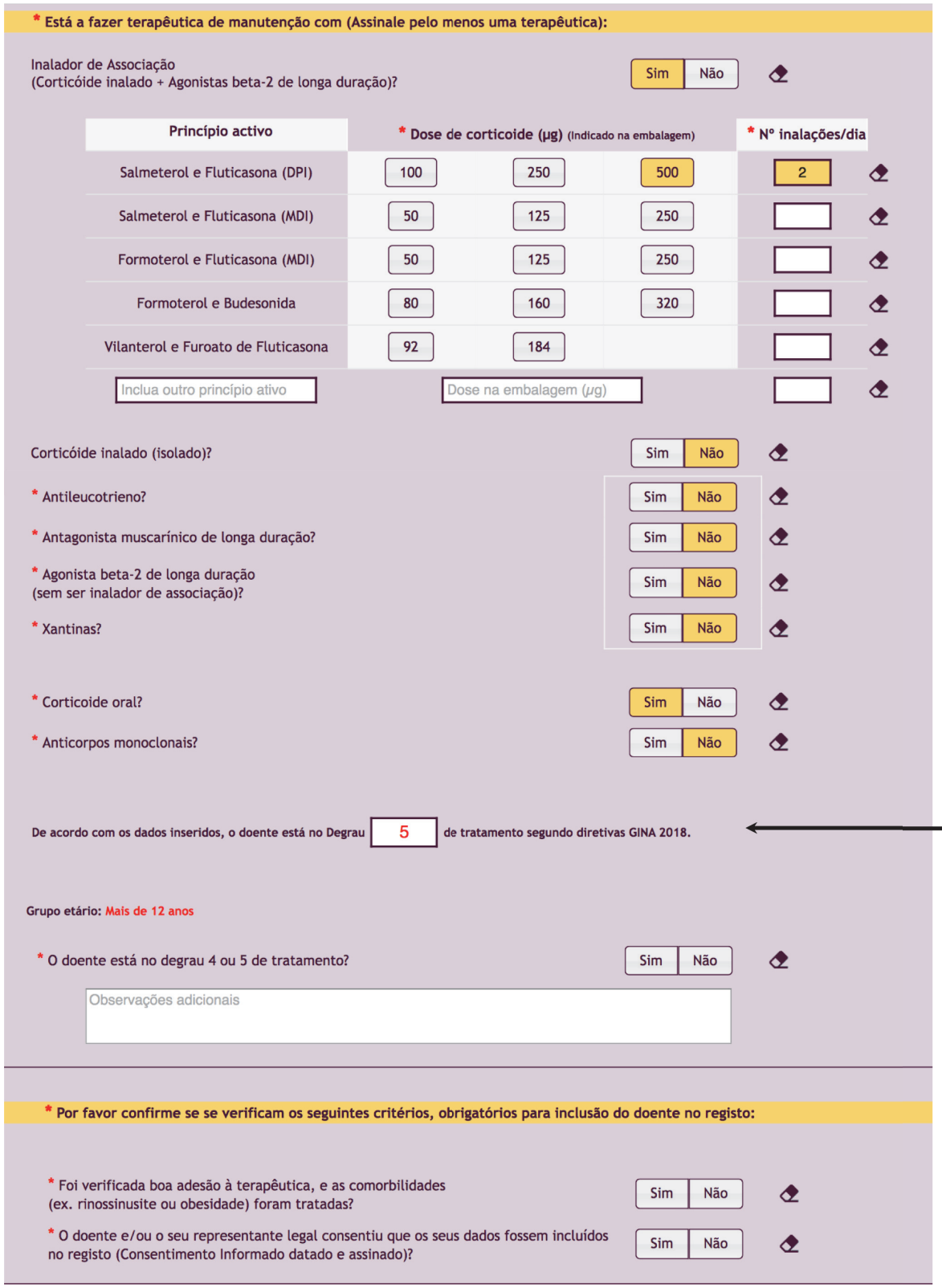

Figure 2: Screenshot of the implemented automatic algorithm to determine the step of treatment, based on asthma medication according to GINA recommendations. A: treatment step calculated by the algorithm; B: the 3 criteria for patients' inclusion.

Table 2: Domains and data elements recorded in the Portuguese Severe Asthma Registry.

Patient data
Demographic data *(gender∗, birth of month∗ and year∗, birthplace, place of residence∗, body mass index calculation∗, education years∗, smoking habits∗, occupation∗, family history of asthma∗ and of asthma-related death∗, personal history of respiratory infections during early childhood∗, environmental exposures)*
Asthma care information *(age at asthma diagnosis∗, age at severe asthma classification∗, first year of specialized asthma follow-up, medical specialty of the attending physician∗)*
Comorbidities∗§
Atopy and Inflammation biomarkers
Atopy *(total serum IgE∗, allergic sensitization∗, type(s) of diagnostic test used to confirm allergic sensitization∗)*
Inflammation biomarkers *(FeNO, blood eosinophils, sputum eosinophils, sputum neutrophils)*
Diagnostic tests
Lung function tests *(FEV1∗, FVC∗, MEF, residual volume, specific airway resistance, carbon monoxide diffusion capacity, bronchial challenge test)*
Imaging *(thorax X-ray∗, thorax CT scan∗, sinus CT scan, bronchial endoscopy, bone densitometry)*
Arterial blood gases
Control and Quality of Life
Asthma-related healthcare utilization due to asthma in previous 12 months (or since the last appointment, when at follow-up visit) *(number of routine primary care medical appointments, routine hospital care medical appointments, non-scheduled medical appointments∗§, emergency service admissions∗§, hospitalizations∗§, intensive care unit admissions, need for mechanical ventilation, school or labor absenteeism)*
Asthma control assessment according to GINA recommendations[1] *(frequency of daytime symptoms∗§, activity limitations due to asthma∗§, any night awakening due to asthma∗§, frequency of use of reliever medications for asthma∗§, respiratory function, number of exacerbations in last year/week∗§)*
Asthma control self-questionnaires *(CARAT∗§ and external link to ACT)*
Quality of life self-assessment questionnaires *(external link to quality of life self-assessment questionnaires)*
Therapy
Asthma medication∗§ *(OCs, ICs, LTRAs, LABAs, SABAs, LAMAs, SAMAs, xanthines, immunosuppressors, immunotherapy, monoclonal antibodies, antibiotics, therapy adherence, inhalation technique)*
Other medication *(proton pump inhibitor, anti-depressive/anxiolytics, intranasal steroids, antihistamines, long-term oxygen therapy, non-invasive ventilation)*

∗Compulsory data elements at initial visit; § compulsory data elements at follow-up.
IgE: immunoglobulin-E; FeNO: Fractional exhaled Nitric Oxide; FEV1: forced expiratory volume in the first second; FVC: forced vital capacity; MEF: midexpiratory flow; CT: computed tomography scan; CARAT: Control of Allergic Rhinitis and Asthma Test [33, 34] and ACT: Asthma Control Test [35]; OCs: Oral Corticosteroids; ICs: inhaled corticosteroids, LTRAs: Leukotriene Receptor Antagonist; LABA: Long-Acting Beta 2 Agonist; SABA: Short-Acting Beta Agonist; LAMA: Long-Acting Muscarinic Antagonist; SAMA: Short-Acting Muscarinic Antagonist.

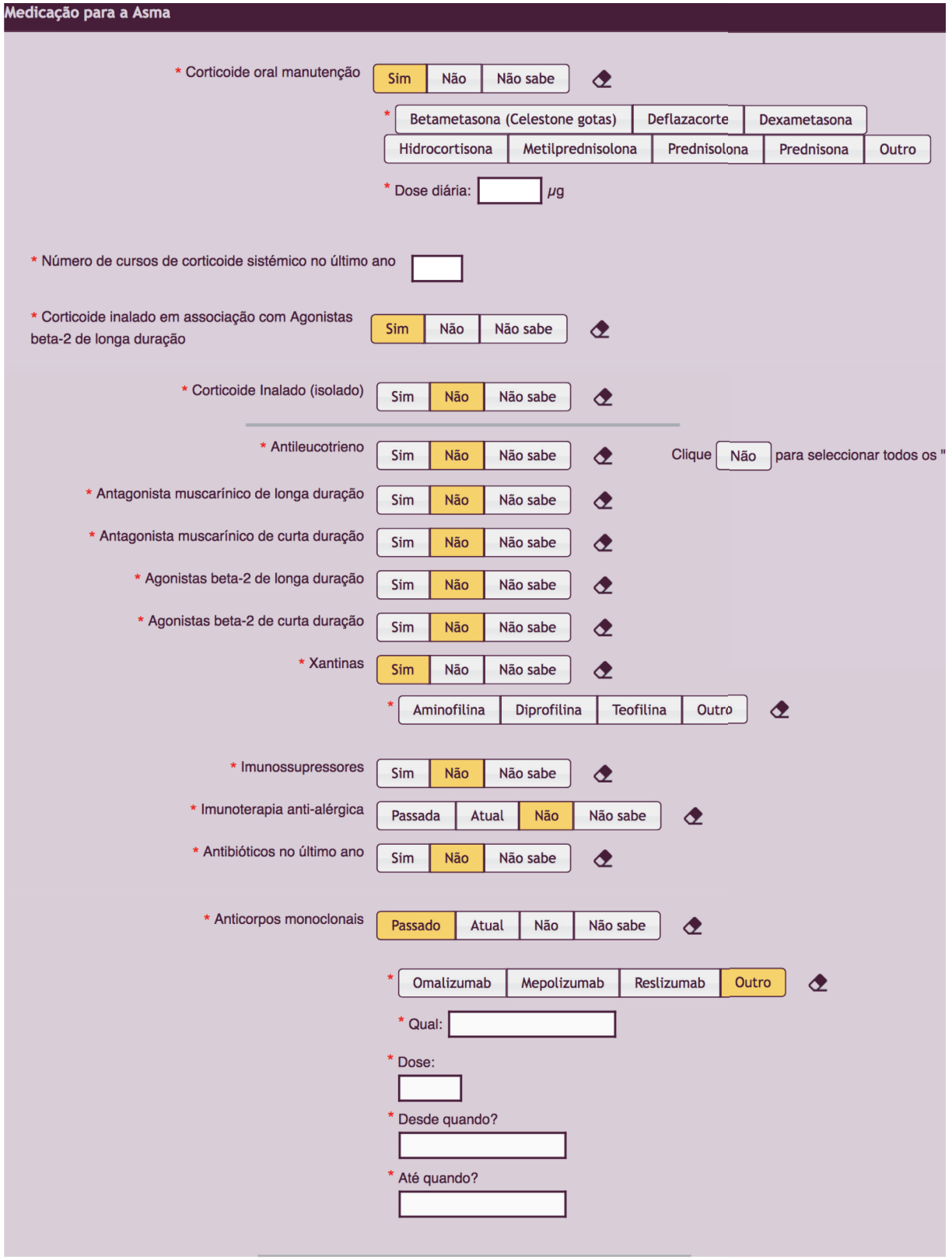

Figure 3: Screenshot of RAG, picturing asthma medication being collected by RAG.

Table 3: RAG features useful to support severe asthma management.

Elements of chronic care management [36]	**RAG features**	
	Current	Future
Ensure regular follow-up	Displays for each physician a list of their patients and date of the last medical appointment	Display a simple message with the counting the months since the last appointment and flag patients without medical review in more than 6 months
Facilitate individual patient care planning	For specified measurements, displays the information inserted in the last appointment and its progress over time	At the beginning of each follow-up appointments, a brief report of the previous appointment will be displayed
Embed evidence-based guidelines into clinical practice	has a decision support tool to identify patients treated in step 4 or 5 according to GINA recommendations	
Monitor the performance of practice team	Displays aggregated data on the number of patients included by each center	Aggregated real-time data with different graphic displays of trends on specified management and clinical outcomes will be produced, to give a feedback to physicians about the status of the care of their patients and/or healthcare center, towards delivering the recommended care for severe asthma.

patients, using a tool consensually defined to be applied prospectively by specialists from Portuguese hospitals, is ambitious but can improve the information on the disease and contribute to the adoption of evidence-based policies for severe asthma care. This harmonized approach is essential to improve the management of the different phenotypes this pathology. The Portuguese registry was designed to enable future linkage with other databases, as registries from other countries, as well as the Portuguese Pharmacovigilance Authority.

The data elements included in RAG were selected to reflect the current clinical status of the patient avoiding unnecessary burden within the clinical workflow. Through a multistep consensus method, a balance was achieved between the data commonly used by clinicians, the data included in other severe asthma registries, the data needed for the RAG's reliability, and the expected overall burden for respondents. Therefore, there was an effort to data collected by RAG which can be compared to data collected by other registries enabling comparisons across populations and settings. A consensus method was used to summarize information from different sources, to gather insights from experts and to enable decision-making [39]. After the selection and implementation of the data elements and validation rules, RAG was pilot-tested and iteratively improved before release.

The patients' inclusion criteria were also defined by consensus and an automatic algorithm was implemented to assist patients' eligibility assessment, based on GINA recommendations. Clinical guidelines provide a link between the best available evidence and the clinical practice, having the potential to improve enormously patient care [40]. However, these may have limitations especially for a particular disease where evidence is still insufficient as in severe asthma and cannot be used as a strict formula. During algorithm development became clear that GINA 2018 treatment steps do not account for all possible therapeutic combinations. In the future, it would be important to assess if clinically relevant combinations are not included in the GINA recommendations, to contribute to the improvement of the recommendations concerning severe asthma.

Disease registries are used to support healthcare providers on disease care and to gather evidence for scientific and policy purposes. Therefore, a disease registry should (1) facilitate the access to patient-specific information at the point of care for healthcare delivery and provide status reports of aggregated information to give feedback to physicians or to medical groups about the patient population [36] and (2) provide real-world data on clinical practice, patient outcomes, safety, and/or comparative effectiveness for research purposes[5]. RAG has several features to support healthcare providers on severe asthma care (Table 3). Additionally, as suggested by the members of REAG, RAG includes the automatic generation of clinical notes based on the inputted data that can be pasted into the institutional electronic clinical record of the patient, avoiding duplication of effort.

Real-world prospective observational research, including long-term follow-up data provided by registries, is increasingly considered important to generate evidence regarding effectiveness, safety, and quality of care [41]. The utility of a

registry relies on the quality of data collection and storage [5]. RAG's data are collected at the time of routine medical appointments, in the same manner for every patient, with specific and consistent data definitions. To minimize errors related to data completeness and consistency, several logical and validation rules have been implemented and periodic data audits are being planned. An additional challenge is the recruitment and retention of participants that is critical to the generalizability of a registry [5]. Potential RAG users were involved from the beginning in the development and implementation process and stated their motivation to include patients. Nevertheless, to retain users' interest, the burden of participation was kept as low as possible and features wanted by the physicians were implemented.

RAG was designed to comply with security and data protection standards, including key challenges of the new European GDPR. No individually identifiable information of the patient is recorded in the database. Only the his/her physician can link the recorded data to the patient that remains the owner of the data. RAG's data sharing policies allow the use of data for research, requiring the consent of the physician that recorded the data and a simple process to gather this consent was implemented.

5. Conclusions

The Portuguese Severe Asthma Registry is a national web-based disease registry of adult and pediatric severe asthma patients. The development and implementation of the RAG was a multistep consensus process. RAG includes automatic assessment of eligibility, easy data input, and features for exporting and sharing data. It allows prospective clinical data collection, promotes standardized clinical records, and creates a secure virtual setting for collaborative clinical research. RAG database is prepared for future data exchange with international databases. In the future, the analysis of RAG data may contribute to inform evidence-based healthcare policies for severe asthma.

Data Availability

Data sharing is not applicable to this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Review Article

Anti-IL-5 and IL-5Ra: Efficacy and Safety of New Therapeutic Strategies in Severe Uncontrolled Asthma

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The current developments of the new biological drugs targeting interleukin 5 (IL-5) and IL-5 receptor allowed to expand the treatment options for severe hypereosinophilic asthma. Clinicians will then be able to choose between antibodies targeting either circulating IL-5 or its receptor expressed on eosinophils and basophils. The available clinical trials consistently reported favorable results about the reduction of exacerbations rate, improvement in quality of life, and sparing of the systemic steroid use, with a favorable safety profile. Two of these new drugs are administered subcutaneously, mepolizumab every 4 weeks and benralizumab every 8 weeks, whereas reslizumab is given intravenously monthly on a weigh-based dose. In the future, the research actions will be involved in the identification of a single biomarker or multiple biomarkers for the optimal choice of biological agents to be properly prescribed.

1. Introduction

The recent change in the definition of asthma, from a unique disease characterized by a reversible airway obstruction to a heterogeneous disease (encompassing numerous phenotypes), prompted the research to look for more detailed pathogenic aspects, as endotypic targets, especially in uncontrolled or severe patients. In this regard, two main different phenotypes/endotypes of asthma could be distinguished, based on their inflammatory characteristics, that are, T helper lymphocyte type 2 (TH2)-high and TH2-low, depending on the predominance of TH2 cytokines [1]. The more and more detailed knowledge of the pathogenic mechanisms led to the discovery of "targeted" treatments to be used in subsets of non-controlled asthmatic patients. For historical and cultural reasons the best known pathogenic mechanism is mediated by eosinophils and IL-5. In fact, within the TH2-high asthma, allergic asthma (early onset, eosinophilic inflammation, and IgE mediated sensitization) remains a paradigm. Two main approaches were evaluated to block the action of IL-5 on eosinophil activation, survival, and migration. The first one is to block the circulating cytokine, and the second is to interfere with the IL-5 receptor alpha on eosinophils. Although the earliest experimental data on the effects of anti IL-5 in asthmatic patients were disappointing, with the only evidence that anti-IL-5 reduced eosinophils in peripheral blood, airways, and bone marrow, but no effects on airway hyperreactivity and bronchial allergen [2–5], a more accurate analysis of the data related to the first studies has allowed to highlight a better response to these drugs by those who had high levels of serum eosinophils. The use of these drugs has therefore been restricted to asthmatic patients with these biochemical characteristics.

The subsequent available clinical trials have shown a good efficacy in the above mentioned selected patients, with a favorable safety profile, for all of the three drugs [6].

2. IL-5 and Its Receptor Alpha

IL-5 is a 13-amino acid protein forming a 52-kDa homodimer, which has long been evaluated as a valuable therapeutic target [22], since it represents the main stimulus for growth, differentiation, survival, and activation of the cells [23]. IL-5, IL-3, and granulocyte-monocyte colony-stimulating factor (GM-CSF) all belong to the β common chain family and are able to bind a receptor involving the interleukin-5Ra and the common β subunit βc [24–26]. While IL-5 is more specifically involved in maturation and activation of eosinophils, IL-3 and GM-CSF have a more broad action, as survival factors for these cells [27]. Recently, IL-33 was found to play a non-negligible role in eosinophils homeostasis, through the activation of innate lymphoid cells type 2 (ILC2) [28].

3. IL-5 Antagonists

The awareness that IL-5 is involved in development, maturation, and action of eosinophils prompted the research to evaluate this cytokine as a possible therapeutic target in severe uncontrolled hypereosinophilic asthma. Two different mechanisms of action were identified, the former acting directly on IL-5 and the latter directly on IL-5 receptor alpha (IL-5Ra). Two different drugs are currently available to block IL-5: mepolizumab (recently commercialized with brand name Nucala; GSK) [2, 23] and reslizumab (proposed trade name Cinqair; Teva). Another biological drug blocking the IL-5 receptor alpha was approved by Food and Drug Administration (FDA) (Benralizumab, Fasenra) [29]. The antagonism to circulating IL-5 is intended to decrease the proliferation, maturation, and survival of eosinophils, whereas the ILR-a antagonism adds an antibody-dependent cell-mediated cytotoxicity (ADCC). Through this activity, essentially mediated by NK cells, Benralizumab can induce a peripheral and tissue destruction of both eosinophils and basophils [8]. The main end point, in clinical trials, regarding anti IL-5 or anti IL-5R drugs, ever was the reduction of exacerbations, the use of oral corticosteroids (OCS), and the effects on quality of life (QoL).

4. General Therapeutic Aspects

First clinical trials about these drugs evaluated the intravenous route of administration with the above mentioned results. After these trials a second route has been evaluated for all of these drugs, the subcutaneous. For mepolizumab and benralizumab, it was shown that both routes were equally effective, with a better safety profile and a more convenient use of the subcutaneous route. The same thing did not happen for reslizumab; indeed recently two phase III studies (evaluating subcutaneous reslizumab, 110 mg) did not meet the primary endpoint: the reduction of exacerbations in patients with severe uncontrolled hypereosinophilic asthma (blood eosinophils >300/mcL) in the first one (NCT02452190) and the reduction of daily systemic steroids in the second (NCT02501629) [30]. Therefore, so far, the optimal administration route for reslizumab remains the intravenous one that, on the other hand, allows to adjust the dose according body weight. Benralizumab is administered subcutaneously, like mepolizumab, at an 8-week time interval. The possibility to choose between two different routes (intravenous or subcutaneous) and a different times of administration (4 or 8 weeks) would allow the clinicians to more properly personalize the therapy according to the characteristics of the drugs and the patients' needs.

5. Exacerbations

The reduction in exacerbation rate and in the dose of systemic corticosteroids is usually the main endpoints in clinical trials, according to the definition of severe asthma [31]. Omalizumab (anti-IgE [32]) remained for 10 years the only biological treatment available for severe allergic asthma. The first regulatory trial with mepolizumab involved 61 subjects with a history of refractory hypereosinophilic asthma and frequent exacerbations. Patients received a monthly dose of 750 mg mepolizumab for one year. There was a reduction of the exacerbation rate in the active arm compared with the placebo group (2.0 vs. 3.4 mean exacerbations per subject; relative risk, 0.57; 95% confidence interval [CI], 0.32 to 0.92; P = 0.02) [33]. In another trial, the efficacy of mepolizumab in reducing exacerbations was tested in 20 adult patients with severe asthma. All patients received 750 mg mepolizumab or placebo for five months. At the end of the study, 12 exacerbations were recorded in the placebo group and two in the mepolizumab group (p=0.008) with a mean duration of exacerbation of 20 weeks in the active group and 12 weeks in the placebo one (P=0.003) [11]. The first trial with exacerbation rate formally defined as primary endpoint was DREAM. Six hundred and twenty-one patients with severe asthma and signs of eosinophilic inflammation were enrolled in this multicentric, double-blind, placebo-controlled trial. Three different intravenous dosages of mepolizumab (75 mg, 250 mg, and 750 mg) and placebo were administered. The exacerbations rate was significantly reduced in the active groups as compared to placebo (48% reduction; <0·0001) [7]. There was no difference in the efficacy and safety among the different doses in order to reduce exacerbations. SIRIUS study, where primary endpoint was the reduction of oral corticosteroids (OCS), evaluated also, as further endpoint, the exacerbations showing a significant reduction (32% less) in patients given mepolizumab compared with placebo [9]. The effects of mepolizumab, 75 mg intravenously or 100 mg subcutaneously, were assessed in the MENSA study. In this study, in the intravenous group the exacerbation rate was reduced by 32%, while in the subcutaneous group the decrease was 53% versus placebo [10].

For Reslizumab, the reduction of exacerbations was assessed in two duplicate, multicenter, double-blind, parallel-group, randomized, placebo-controlled (DBRPC) phase 3 trials. The drug (or placebo) was given at 3.0 mg/kg intravenously every 4 weeks for 1 year. The trial reported a significant reduction in asthma exacerbations in the active group (study 1: RISK ratio [RR] 0.50 [95% CI 0.37–0.67]; study 2: 0.41[0.28–0.59]; both p<0.0001). In addition, the time to first exacerbation was considerably longer in the active than in the placebo group [13].

Similarly to mepolizumab and reslizumab, several studies with Benralizumab evaluated the exacerbation rate reduction as primary endpoint. The results of a phase II DBRPC showed a reduction of exacerbations. A significant reduction of exacerbations rate (49%) and exacerbations requiring hospitalization (60%; 1.62 vs 0.65; P=.02), was also reported in another trial (3.59 vs 1.82; P=.01[18]). The SIROCCO study was a double-blind, parallel-group, placebo-controlled phase 3 clinical trial, where patients were assigned to 400 every four weeks and 398 every eight weeks Benralizumab 30 mg or placebo subcutaneously. The active drug reduced the asthma exacerbation rate, during the year of observation both in the 4-week (RISK ratio 0.55, 95% CI 0.42–0.71; p<0.0001) and in the 8-week group (0.49, 0.37–0.64; p<0.0001) [19]. Exacerbation reduction has been evaluated also in CALIMA study, with the same inclusion criteria and dosing regimens of SIROCCO, showing similar results with a significantly lower annual exacerbation rate both in the group treated with 30 mg every 4 weeks (0.60 [95% CI 0.48–0.74], rate ratio 0.64 [95% CI 0.49–0.85], p=0.0018, n=241), and in the one treated every 8 weeks, compared with placebo [20]. The latest published clinical trial on Benralizumab in severe hypereosinophilic patients (ZONDA) reported a significant reduction in exacerbation rate in both groups (30 mg/4 weeks or 30 mg/8 weeks), with a decrease of 55% in patients treated every 4 weeks, and 70% in those who assume therapy every 8 weeks, versus the one treated with placebo [21].

6. The OCS Sparing Effect

A special attention was recently devoted to steroid-dependent patients; this was due to the well-known burden of steroid related side effects (diabetes, hypertension, obesity, cataract, etc.) [34]. In one study, all enrolled patients received a mean daily dose of 10 mg of prednisone both in placebo and in mepolizumab group. After the treatment period, the active group had a mean reduction of their dose of 83.8 ± 33.4%, as compared to 47.7 ± 40.5% in the control group (P=0.04) [11]. In the SIRIUS study, in a cohort of 135 patients with severe eosinophilic asthma those receiving mepolizumab could reduce the dose of oral steroids 2.65 times versus those receiving placebo (95% CI, 1.25 to 4.56; P=0.008) [9]. A trial where reslizumab's OCS sparing effect has been indicated as primary endpoint is actually ongoing (NCT02501629). Preliminary results of this trial have been recently published in an official note, showing the failure of the drug in order to reduce daily OCS dose [30]. The effect of Benralizumab, on the reduction in the OCS dose, has been recently published. The study design involved 28 weeks of Benralizumab (30 mg subcutaneously, either every 4 weeks or every 8 weeks [with the first three doses administered every 4 weeks]) versus placebo. For both active groups, the median OCS reduction at week 28 was 75% in active patients compared with 25% in the placebo group. The percentage of patients that could completely withdraw their OCS daily dose (secondary endpoint) was 56% in the every 4 weeks and 52% in the every 8 weeks administration, as compared with 19% in the placebo group [21].

7. Quality of Life (QoL)

In addition to exacerbations, lung function, and safety, the effects on QoL are also relevant when a new drug is evaluated. Within the above mentioned trials with mepolizumab, Haldar et al. evaluated the effect of the medication on QoL, measured by the Asthma Quality of Life Questionnaire (AQLQ). After treatment, AQLQ improved from 0.55 in the active group to 0.19 [33]. On the other hand, the DREAM study failed to demonstrate a statistically significant effect on FEV_1 and AQLQ [7]. MUSCA is the most recent large trial assessing health-related quality of life (HRQOL) in severe asthmatic patients as primary endpoint. It is a randomized, double blind, placebo-controlled, parallel group, multicenter, phase 3b trial, with 274 mepolizumab patients and 277 placebo patients enrolled. Inclusion criteria were a history of at least two exacerbations in the previous year treated with corticosteroids. The St George's Respiratory Questionnaire (SGRQ) was used to assess the changes in HRQOL. At week 24 a significant improvement in symptoms in the active group was documented as compared with placebo [12]. One of the first trials with reslizumab evaluated the effect of 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, or 1.0 mg/kg or placebo, in severe asthmatic patients with persistent symptoms, needing OCS and high-dose of inhaled steroids. With the 1 mg/kg dose a decrease of peripheral eosinophils was seen, but no improvement in symptoms [35]. A more recent study demonstrated a significant reduction in ACQ-7 in patients treated with reslizumab vs placebo (71% vs. 57%; p=0.01) [15]. In a similar trial, where reslizumab was given at 0.3 mg/kg and 3 mg/kg, an improvement in QoL, measured with ACQ, ACQ-5, ACQ-6, and AQLQ, was demonstrated with the highest dose [16]. Concerning Benralizumab, an improvement in QoL (ACQ-6 and AQLQ) was seen, especially in subjects with baseline blood eosinophils ≥300 cells per μL [19, 20]. An improvement in QoL was confirmed also in the ZONDA study, where active arm ACQ-6 scores decreased by 0.55 points vs. placebo (p=0.001) [21] (Table 1).

8. Safety

The general safety of anti-IL biologicals, as assessed in controlled trials, has been described and reviewed elsewhere [36, 37]. Nonetheless, other special safety aspects have been proposed as a matter of discussion.

For instance, the defensive role of eosinophils, especially against helminthic infections, is well known, and for this reason the effects of the drug-induced depletion of eosinophils were debated. Indeed, several studies in guinea pigs treated with eosinophils antiserum failed to demonstrate an increased risk of helminth infestation [38]. Also, the long term (more than 6 months) treatments in mice and primates with antibodies abating eosinophils did not demonstrate any observable adverse effects [39, 40]. The most common non-serious AE in clinical trials with mepolizumab were injection site reaction, headache, nasopharyngitis, and upper respiratory tract infection, not different from placebo groups [7, 9–12, 33]. In the largest clinical trials, some serious adverse events (SAE) were described, mainly worsening

Table 1: Exacerbations, OCS sparing, QoL, and safety of main clinical trials about anti-IL-5 and anti-IL-5Ra.

		EXACERBATION	OCS sparing	QoL	SAFETY
MEPOLIZUMAB	Pavord et al. [7]	exacerbation (48%with 75mg dose/39% with 250mg dose/52% with 750mg dose)	not performed	no improvement of QoL (tests using AQLQ)	common: headache, nasopharyngitis, infusion related reaction Serious: 3 death (1 septic shock after acute pancreatitis, fatal asthma attack, suicide)
	FloodPage et al. [8]	no significant difference between groups (16% placebo, 18 % 250 mg, 10% 750 mg)	not performed	improvement in treated patients, with all dose	serious: placebo (bladder carcinoma, unintended pregnancy, and asthma exacerbation); 250 mg of mepolizumab (hydrocephalus/cerebrovascular disorder, constipation, and gastrointestinal disturbance); 750 mg of mepolizumab (asthma exacerbation)
	Bet et al. [9]	32% exacerbation less	reducing daily dosage (2,65 times more than patient receiving placebo)	small change in ACQ	common: headache, nasopharyngitis, infusion related reaction Serious: asthma exacerbation, pneumonia (both in placebo group)
	Ortega et al. [10]	exacerbation (with intravenous medication, 47%; with subcutaneous administration, 53%)	not performed	improvement in QoL	common: headache, nasopharyngitis, upper respiratory tract infection
	Nair et al. [11]	reduction of exacerbations in treated patients	reducing daily dosage	not performed	common: 1 patient with shortness of breath, 1 with aches and tiredness serious: 1 death in placebo group
	Chupp et al. [12]	reduction of exacerbations of 58%	not performed	improvement of QoL (tests using SGRQ)	common: headache, nasopharyngitis, urticaria, arthralgia, arrhythmias, injection-site reaction Serious: 8 arrhythmias (2 in mepolizumab and 6 in placebo), 1 deep venous thrombosis in mepolizumab group

Table 1: Continued.

		EXACERBATION	OCS sparing	QoL	SAFETY
RESLIZUMAB	Castro et al. [13]	exacerbation (people without exacerbation: 44%with placebo, 61% with reslizumab)	no improvement in OCS sparing	improvement of QoL (test using ACQ)	common: nasopharyngitis, upper respiratory tract infection serious: 2 anaphylactic reaction
	Castro et al. [14]	exacerbation (people without exacerbation:52% with placebo,73% with reslizumab)	no improvement in OCS sparing	improvement of QoL (test using AQLQ and ACQ-7)	common: nasopharyngitis serious: pneumonia, worsening of asthma
	Corren et al. [15]	not performed due to the short observation period (16 weeks)	not performed	improvement of QoL (test using ACQ-7)	serious: 2 anaphylactic reactions, 1 colon cancer (all in reslizumab group)
	Bjemer et al. [16]	not performed due to the short observation period (16 weeks)	not performed	improvement of QoL (test using ACQ and AQLQ)	serious: *placebo (*1 acute myocardial infarction), 3 asthma exacerbations, 1 sinusitis, 1 pneumonia, 1 road traffic accident and 1 rib fracture
BENRALIZUMAB	Castro et al. [17]	no difference in noneosinophilic patients between benralizumab and placebo. Reduction in eosinophilic patients.	not performed	improvement in AQLQ in people with at least 300 eosinophils/mmc	serious: 100 mg dosage acute cholecystitis, herpes zoster, polyarteritis nodosa, and uterine leiomyoma 20 mg dosage: erythema nodosum
	Nowak et al. [18]	exacerbation (49%) and exacerbation requiring hospitalization (60%)	not performed	no significant improve in ACQ and AQLQ	common: headache, asthma, dizziness, cough, pyrexia, bronchitis, anxiety, muscle spasm serious: tachycardia and anxiety
	Bleecker et al. [19]	exacerbation in Q4W and Q8W	not performed	improvement in patients with baseline	common: nasopharyngitis, worsening of asthma serious: allergic granulomatous angiitis, panic attack, paraesthesia
	Fitzgerald et al. [20]	exacerbation in Q4W and Q8W	not performed	blood eosinophils ≥300 cells per μL	common: nasopharyngitis, worsening of asthma serious: urticaria, asthma, herpes zoster, chest pain
	Nair et al. [21]	exacerbation (55% with 30 mg dose every 4 weeks; 70% with 30 mg dose every 8 weeks)	interruption of OCS (56% of who received drug every 4 weeks and 52% of 8 weeks administration, as compared with 19% treated with placebo)	improvement in patients with baseline	serious: worsening of asthma, pneumonia, hearth failure, pericarditis (placebo). Two case of death in Q8W due to pneumonia and acute cardiac failure.

of asthma [5, 9]. Three fatal events, all in the intravenous mepolizumab groups, were reported, but none of these cases were considered as drug-related [7]. No fatal event was reported with the subcutaneous route. With reslizumab, four cases of anaphylactic reaction were described in two different trials [13, 15]. Also for reslizumab the main SAEs were worsening of asthma, followed by pneumonia [10, 13, 14, 16]. One patient in the placebo group died due to multiple-drug overdose [13]. Worsening of asthma appeared as the most frequently described SAE also in the benralizumab studies [20, 21]. In those trials, some fatal events (due to pneumonia, acute cardiac failure, cerebral hemorrhage, asthma, opioid overdose, suicide, road traffic accident, acute myocardial infarction, colon neoplasm, and unknown causes) were in the active group patients. Pulmonary embolism, myocardial infarction, and unknown causes were in patients treated with placebo [17, 19–21].

9. Anti-IL-5 Treatments: Practical Aspects and Problems

All the available (or soon available) IL-5 antagonists (mepolizumab, reslizumab, and benralizumab) show a favorable cost-to-benefit profile, in addition to clinical efficacy and biological effects. When all drugs will be marketed, we could choose between different kinds of administration (intravenous or subcutaneous) and administration frequency (4 or 8 weeks). For administration route, it has been already said that reslizumab, at least at the moment, remains with the only intravenous way; regarding the frequency of administration it is interesting that benralizumab could be dispensed every 8 weeks. In published clinical trials and documents no pathophysiological motivation was provided to explain the possibility to doubling administration time; however it is evident that, with the same efficacy results shown in the CALIMA and in the SIROCCO study [19, 20] at 4-week and 8-week administration, the pathway every 8 weeks is economically and operationally more sustainable for this drug.

Regardless of the route and frequency of administration, in the main clinical trials all the above mentioned IL-5 antagonists have proven to be more effective in the severe form of asthma with high levels of blood eosinophils (300 cells/mm^3 for Benralizumab and mepolizumab and 400 cells/mm^3 for reslizumab). The fact that it has been proven that all three drugs are more effective in the same types of subjects (severe asthmatics with serum hypereosinophilia), once on the market, could be a problem. Indeed if we have three drugs with similar patient targets, and a very similar efficacy between the different molecules, it will be difficult to choose [41]. Moreover, regarding efficacy, it has been shown that all anti-IL-5 drugs not only need high blood eosinophils levels, but also highlight the fact that the number of eosinophils present in patients' serum correlates with the effect of the drug administered. Indeed a secondary analysis of MENSA and DREAM studies demonstrates that the reduction in exacerbations rate is positively associated with increasing blood eosinophil count at baseline [42].

Nevertheless, the clinical aspects (symptoms, pulmonary function, exacerbations, and exhaled nitric oxide) still perceived the only (and insufficient) predictive biomarkers to guide the prescription of such expensive drugs. The coexistence of chronic rhino sinusitis with nasal polyposis could be a criterion for the choice of one drug or other biological drugs. Also, the route of administration (intravenous or subcutaneous) and the possibility of adjusting the dosage would be possible suggestions for clinicians. In addition to IL-5 antagonists, other biological drugs such as anti IL-4 and IL-13 [43] were proposed, although the recent preliminary results on Tralokinumab (anti IL-13) displayed unfavorable results (STRATOS 2 (exacerbations) and TROPOS reduction in OCS use) in severe asthma [44]. The possible answer is biomarkers, some biological or clinical samples, able to drive clinician to the choice [45]. Notwithstanding some studies proposed several biomarkers, such as serum total IgE levels (IgEs) [46], FeNO, blood, and sputum eosinophil count [47, 48]; there is not a certain role of these samples as predictive indicator of response for one or the other drug. Other biological samples have been evaluated, like periostin, both in bronchoscopy biopsies [49] and in less invasive way [50], and are still under evaluation. Given that clinical trials have shown promising efficacy for all three drugs described, as already stated, once all these drugs are marketed the challenge could be which one to choose to provide an increasingly personalized medicine and choose the one that preventively could be the best. At the moment, due to the fact that no certain biomarker has been discovered, to choose the better drug for our patients, we could use a more clinic approach and we can rely on what emerged from trials and literature. Several authors suggest that the single dosage of mepolizumab could be a limit in overweight-obese patients, and the possibility to a weight-adjustment could be useful. About that Mukherjee and coauthors have described a trial where ten patients, demonstrating a non-fully response to mepolizumab after 1 year of administration, after a 1-year period of wash-out, have been treated with 3.0 mh/Kg of reslizumab with an increase of QoL and decrease of sputum and blood eosinophilia after 4 months of administration [51]. This could be used as a discriminant to choose one drug rather than another. On the other hand mepolizumab seems to have the same efficacy in the patients treated in the trials both at the marketed dosage (100 mg) and at higher doses, confirming its effectiveness regardless of weight, making it safe and effective to be prescribed independently of the body mass index (BMI) value [7, 10]. Regarding the anti-receptor drug, benralizumab, an advantage could be the periodicity of administration; indeed, after a "run-in" period where for three months the dosage is at 4-week frequency, the drug will be injected every 8 weeks. This therapeutic scheme could be advantageous due to the fact that the intake of the drug with a 8-week frequency would decrease the indirect costs (lost work days, visits made, etc.), and, depending on the cost of the drug agreed upon with the local health ministries, also the direct ones. We could have greater clarity on the choice of drugs with the development of single biomarkers or panels of laboratory and clinical parameters, and real life studies.

10. Conclusions

The wide variety of anti-IL-5 antagonists or IL-5 receptor blockers allow to have alternative treatment options for patients with severe hypereosinophilic patients. All the three drugs herein reviewed displayed a good safety profile, and a favorable clinical efficacy in the selected patients. It remains true that we do not still have reliable predictive markers to detect which single patient will respond individually to each of such expensive treatments. Also, the different routes of administrations would provide clinicians with the opportunity to choose the drug according to drug characteristic and patient's needs. At present, the best biomarker in patient eligible for anti-IL-5 or IL-5ra is blood eosinophils, exhaled nitric oxide, and clinical phenotyping (age of onset of asthma, atopy, and presence of nasal polyposis). Predictive biomarkers allowing a better prescription of a personalized medicine are needed, although the introduction in clinical practice of novel biologics targeted to severe asthma represents a step forward.

Conflicts of Interest

Giovanni Passalacqua and Giorgio Walter Canonica were consultants/speakers for ALK-Abellò, AstraZeneca, Lofarma, Novartis, and Stallergenes-Greer.

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Review Article

Eosinophils Target Therapy for Severe Asthma: Critical Points

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Asthma is a chronic and heterogeneous disease, which is defined as severe disease whenever it requires treatment with a high dose of inhaled corticosteroids plus a second controller and/or systemic corticosteroids to prevent it from becoming "uncontrolled" or if it remains "uncontrolled" despite this therapy. Severe asthma is a heterogeneous condition consisting of phenotypes such as eosinophilic asthma, which is characterized by sputum eosinophilia, associated with mild to moderate increase in blood eosinophil count, frequently adult-onset, and associated with chronic rhinosinusitis with nasal polyps in half of the cases. Eosinophilic asthma is driven by T2 inflammation, characterized, among the others, by interleukin-5 production. IL-5 plays a key role in the differentiation, survival, migration, and activation of eosinophils, and it has become an appealing therapeutic target for eosinophilic asthma. In recent years two monoclonal antibodies (mepolizumab and reslizumab) directed against IL-5 and one monoclonal antibody directed against the alpha-subunit of the IL-5 receptor (benralizumab) have been developed. All these IL-5 target drugs have been shown to reduce the number of exacerbation in patients with severe asthma selected on the basis of peripheral blood eosinophil count. There are still a number of unresolved issues related to the anti-IL5 strategy in eosinophilic asthma, which are here reviewed. These issues include the effects of such therapy on airway obstruction and asthmatic symptoms, the level of baseline eosinophils that predicts a response to treatment, the relationship between blood and airway eosinophilia, and, perhaps most importantly, how to elucidate the pathogenetic role played by eosinophils in the individual patient with severe eosinophilic asthma.

1. Introduction

Asthma is a chronic disease characterized by different clinical presentations, comorbidities, and outcomes, affecting an estimated 300 million people worldwide, of all ages, who usually need many specialists in order to be well managed [1–5]. Although asthma is generally mild and well controlled, the severe form, which represents at most 10% of asthmatic patients, can be refractory to conventional therapies, such as inhaled corticosteroids (ICSs), inhaled bronchodilators, and oral leukotriene modifiers [6, 7].

The outcome of asthma therapy becomes very important in terms of public health, social impact, and quality of life, particularly for those people suffering from severe asthma. It is therefore becoming more and more important to identify patients' phenotypes and to target precise molecules to obtain a good asthma control.

2. Asthma Phenotypes and Endotypes

Asthma can be classified into different phenotypes, according to its clinical presentation, concomitant comorbidities such as nasal polyposis or obesity, identifiable triggers, including allergen or aspirin sensitivity and response to therapy. Phenotypes, as measurable and observable features of asthma, are also available in defining eosinophilic and noneosinophilic asthma. In fact, the lack of knowledge of pathogenesis underlying each different phenotype represents a limit in understanding the mechanisms of Asthma subgroups and in disease management. Recently it has been proposed that

the definition of endotype represents a specific biological mechanism which underlies a given phenotype.

The identification of different endotypes provides a contribution to lead novel treatments, such as biologic therapies to target specific inflammatory mediators (e.g., IL-5) [8, 9].

The two-main recognized asthma endotypes are based on high or low T-helper 2 (TH2) cell airway inflammation. Considering also type 2 innate lymphoid cells (ILC2), which are outside the originally described Th2 cell population but producing the same cytokines, (T2)-high or (T2)-low has emerged as a more appropriate and inclusive term for defining asthma endotypes.

The pathophysiology of T2 low asthma is not completely understood, but it is thought that it could be characterized by neutrophilic inflammation, suggesting a TH1 and/or TH17 cells activation.

On the other hand, in T2 high asthma, overproduction of eosinophils, driven by an overproduction of type 2 cytokines from T-helper 2 and innate lymphoid cells, is commonly found in many patients, and it correlates with more severe disease, with airway dysfunction [10].

3. Eosinophilic Asthma and IL5

In more than 50% of patients affected by severe eosinophilic asthma (SEA), both blood and sputum eosinophilia are associated with worse disease control and prognosis [11]. In addition, blood eosinophilia often reflects asthma severity [12] and the relationship between the reduction in sputum eosinophils and the reduction of exacerbations after ICS therapy is well recognized [13].

Interleukin 5 (IL-5) is a cytokine produced by limited types of cells, such as CD4+ T cells, innate lymphocytes type 2 (ILC-2), mast cells, and eosinophils, which are all involved in the airway inflammation of asthma. Whatever the source, IL-5 plays a major role in the differentiation, survival, migration, and activation of eosinophils. This is the reason why IL-5 represents an appealing therapeutic target for hypereosinophilic conditions.

4. Anti-IL5 Strategy in Eosinophilic Asthma

At the beginning of 2000s, the therapeutic role of IL-5 antagonists in asthma was postulated following the observation in rats of the eosinophils reduction in BAL and lung tissue and reduction of airway hyperresponsiveness after treatment with anti-IL5 monoclonal Antibodies (mAbs) intranasally, intravenously, or intraperitoneally, suggesting a good outcome also in treatment of human asthma [14].

Leckie et al. analyzed the effects of mepolizumab, an anti-IL5 monoclonal antibody, in 24 patients with mild asthma, observing a reduction of eosinophils in sputum and blood after allergen challenge, but they did not find a decrease in airway hyperresponsiveness to histamine or in late reaction after allergen challenge [15]. A few years later, it was observed that mepolizumab induced reduction in blood eosinophilia and a slight improvement in FEV1 in asthmatic patients taking high doses of ICS and/or oral corticosteroids, without significant changes in other clinical outcomes [16].

The efficacy of mepolizumab in patients with eosinophilic asthma has been preliminary reported in in 2009 in 2 randomized double-blind, placebo controlled studies. In the first one, Haldar demonstrated the reduction of exacerbations and the improvement in AQLQ scores in 29 patients with refractory eosinophilic asthma. The second study by Nair and Coll reported the reduction of eosinophils in blood and sputum, as well as prednisone sparing in 9 patients who had asthma with sputum eosinophilia despite prednisone treatment. In both studies patients received 750 mg intravenously of mepolizumab for 12 and 5 months, respectively [17, 18].

Later, in 2012 another study reported the efficacy of mepolizumab in a group of patients affected by eosinophilic asthma [19]. These observations placed the basis for the selection of patients based on the disease phenotype to achieve a tailored therapy. Furthermore, the knowledge of eosinophils involvement in asthma and the potential to block IL-5 stimulated other research studies to better identify the field of application of the new anti-IL5 mAbs [20].

In the last two years two similar biologics therapies targeting IL-5, mepolizumab and reslizumab, have been approved, as well as anti-IL-5 alpha receptor, benralizumab. These agents can be used as add-on therapy in subjects with an eosinophilic asthma phenotype, poorly controlled with standard therapy. Mepolizumab and reslizumab both target and bind to IL-5 directly, whereas benralizumab targets the IL-5 receptor alpha subunit. [21, 22].

The primary outcome in mepolizumab registration studies was the reduction of annual frequency of significant asthma exacerbations, which was defined as worsening of asthma which needed to be treated with systemic glucocorticoids for at least 3 days or when the patient visited an emergency department or was hospitalized. Secondary endpoints were the effects of treatment on blood eosinophil counts, asthma control evaluated by ACQ-5 score, asthma-related quality of life, and forced expiratory volume in 1 s (FEV1) [19]. Due to the hierarchical gatekeeping in the study design, the secondary endpoints were not analyzed in the registration study, not having reached the significant difference in reduction of exacerbation requiring ED admission between iv mepolizumab and placebo [23].

On the other side, primary outcome for reslizumab registration studies was the change from baseline in pre-bronchodilator FEV1 over 16 weeks. Secondary endpoints included ACQ scores, FVC, forced expiratory flow at 25% to 75% of FVC (FEF25%-75%), patient-reported control of asthma symptoms, short-acting β-agonist (SABA) use in the three days before the visit, blood eosinophil levels, and safety [24]. All the secondary endpoints were reached except for ACQ, which did not show any difference between reslizumab and placebo.

Lastly, the primary outcome in benralizumab registration studies was annual exacerbation rate ratio versus placebo for patients receiving high-dosage ICS plus LABA with baseline blood eosinophils 300 cells per μL or greater (intention-to-treat analysis), while secondary efficacy endpoints were prebronchodilator FEV1 and total asthma symptom score for patients receiving high-dose inhaled corticosteroids plus LABA with baseline blood eosinophils 300 cells per μL or

greater. Additional secondary endpoints were time to first asthma exacerbation, annual rate of asthma exacerbations associated with emergency department visit, urgent care visit, or admission to hospital (defined as an admission to an inpatient facility and/or evaluation and treatment in a health-care facility for 24 hours or longer), postbronchodilator FEV1, ACQ-6 score, and AQLQ(S)+12 score. The annual rate of asthma exacerbations requiring an ED admission did not differ between the benralizumab and placebo, and benralizumab treatment did not alter the time to first asthma exacerbation requiring an emergency department visit or admission to hospital [25].

Analyzing the efficacy studies of the three Anti-IL5 mAbs, it is important to focus on the primary outcome, which is, for mepolizumab and benralizumab [19, 21], the reduction in annual asthma exacerbation numbers and, limited to reslizumab, the improvement in lung function test [24].

The study design for mepolizumab considered only patients with at least two exacerbations in the last year, showing a significant reduction rate in exacerbation of 53% for the group receiving subcutaneous mepolizumab [19], with an exacerbation rate of 0.93/year.

A secondary (post hoc) analysis of data from two randomized, double-blind, placebo-controlled studies of at least 32 weeks duration, DREAM and MENSA, was performed to evaluate the relationship between baseline eosinophil counts and efficacy of Mepolizumab, stratifying patients by different baseline eosinophil thresholds (count and ranges) in the blood, specifically baseline ≥150, ≥300, ≥400, and ≥500 cells per μL, and baseline blood eosinophil ranges (<150 cells per μL, ≥150 cells per μL to <300 cells per μL, ≥300 cells per μL to <500 cells per μL, and ≥500 cells per μL).

It was observed that the exacerbation rate reduction with mepolizumab versus placebo increased progressively from 52% in patients with a baseline peripheral eosinophil count of at least 150 cells per μL to 70% in those with a baseline count of at least 500 cells per μL. When the baseline eosinophil count was less than 150 cells per μL, the efficacy of mepolizumab was clearly reduced [26].

5. Unresolved Issues Related to the Anti-IL5 Therapy in Eosinophilic Asthma

5.1. Annual Exacerbation Rate Reduction. One important question is the clinical meaning of 50% reduction of annual exacerbation rate and the primary outcome of mepolizumab studies, in the patients who report just two exacerbation/year.

Even if exacerbation as defined in clinical trials is an "all-or-nothing" parameter, it is well recognized that patients with high symptom burden have more frequent exacerbations. The decrease of annual exacerbations per se does not automatically imply a comparable decrease of daily asthmatic symptoms, even if a 50% decrease in asthmatic symptoms has been reported in the same registration studies.

Other clinical endpoints, in addition to annual exacerbation rate, as the need of frequent oral corticosteroid use, asthmatic symptoms and quality of life, should be taken into account by clinicians who consider anti-IL-5 therapy for their patients with severe asthma.

5.2. FeNO as a Marker of T2 Inflammation. Outcomes which have been explored in secondary registration studies were the reduction in blood eosinophil count, the improvement in quality-of-life, the increase of forced expiratory flows, and the reduction in using of SABA. Markers of tissue eosinophilia which have been investigated in these studies were the sputum eosinophilia and, in one study only, the change of exhaled nitric oxide (FeNO) after mepolizumab therapy.

FeNO measurement is esteemed to be particularly useful to identify a high T2 state. The official ATS clinical practice guideline recommends that high FeNO > 50 ppb in adults and > 35 ppb in children can be used to indicate eosinophilic inflammation [27] in subjects, not in steroid therapy.

However, in spite of the expected correlation between the FeNO values and the number of eosinophils in the sputum of patients with eosinophilic asthma, in clinical setting a discrepancy is often observed between the two aforementioned values: high FeNO values may be sometimes observed in patients with normal sputum eosinophils count and *vice versa*.

Mepolizumab has been shown to decrease consistently blood and sputum eosinophil counts in patients with eosinophilic asthma, with no effect on FeNO levels [17].

A possible explanation of the absence of efficacy of mepolizumab on FeNO is that the molecular pathways that lead to an increase in FeNO are different from those that underlie the recruitment and activation of eosinophils, as the FeNO is mainly related to the pathways involved in T2 mediated asthma, while peripheral eosinophilia in asthmatic patients depends also upon the activity of lymphoid cells (ILC2) type 2 [28].

Recent evidence shows indeed that, in atopic asthma, the production of FeNO is stimulated by proinflammatory T2-cytokines, other than IL-5, such as IL-4 and IL-13, making NO a biomarker of T2-driven inflammation [29], which is not susceptible to the action of the anti-IL5 mAbs [30].

5.3. Eosinophil Count for the Assessment of Anti-IL5 Efficacy. Peripheral blood and sputum eosinophil counts have been shown to be consistently decreased by anti-IL5 drugs in all the three registration studies [23–25].

It is well known that both blood and pulmonary eosinophils are increased in patients with eosinophilic asthma. Whether peripheral blood eosinophils mirror bronchial tissue eosinophilia is not known. Also how important is the pathogenetic role played by eosinophils in asthma is not completely known. Bronchial eosinophilia may persist even when peripheral blood eosinophil count has been reduced by anti-IL-5 treatment [31].

Even the reduction of bone-marrow eosinophils, obtained by benralizumab treatment, was not able to abolish eosinophils infiltration in bronchial biopsies [13] or ECP levels in the sputum [32]. This observation suggests the important role of local mechanisms and/or of other cytokines in promoting eosinophils priming, recruitment, activation, and survival in the tissues. Nevertheless, as the source of eosinophil in the tissue is from the blood it is conceivable as a cumulative benefit on tissue eosinofil level with persistent

blockade of blood eosinophils, but the duration of blockade required for measurable benefit on tissue eosinophils has not been evaluated.

The best predictor of response to ICS/OCS in patients with airway diseases, not only asthma, but also COPD and chronic cough of eosinophilic bronchitis, is the presence of eosinophils into the bronchial tissue, which is also predictive of response to therapies that indirectly target eosinophils such as anti-(IL-5) monoclonal antibodies [33, 34]. Whether blood or sputum eosinophils levels are the best predictor of therapy response need to be assessed in specific studies.

Any asthma therapy which had eosinophils as target will be much more effective the more it decreases airway eosinophils and the more airway eosinophils are primary players of airway inflammation. Unfortunately, markers of airways eosinophils activation are not currently available, and this is probably a great limitation to identify the asthmatic patients who could benefit more from anti-IL5 therapies.

Free eosinophil granules (FEGs), released after eosinophils' lysis, are detectable in sputum of patients with uncontrolled and severe asthma and the measure of sputum FEGs could be a new marker of eosinophilic airway inflammation [35].

FEGs contain toxic proteins which are responsible for bronchial epithelial damage, and their presence in the sputum is the consequence of eosinophils degranulation, which is an important mechanism of tissue damage driven by eosinophils [33, 36]. Moreover, the release of eosinophils' peroxidase (EPX) has been related to local airway autoimmunity, following the production of anti-EPX antibodies. This autoimmune mechanism has been related not only to failure of mepolizumab therapy but even to worsening of asthma control in some patients with severe eosinophilic asthma [37, 38] who receive the standard dose of mepolizumab (100 mg s.c.). The same patients had been shown to respond to higher dose or to i.v. reslizumab [18, 23].

It has been suggested that lower doses of anti-IL-5 drugs might not neutralize completely IL-5, which could still be able to promote local airway eosinophilia, in spite of the normalization of the blood eosinophil count [39, 40].

Another possibility is that a lower dose of anti-IL5 Mab could drive airway eosinophilia through the production of immune-complex and/or complement activation [38]. Such immune complexes could act as depot of IL5, leading to an increase in biological activity of the bound IL-5 [41]. This is a theoretical risk that has not been corroborated by clinical studies.

It has been recently demonstrated [42] that levels of Ig-bound IL-5 in the sputum of mepolizumab nonresponder patients was associated with increase in sputum eosinophils count.

In conclusion, anti-IL-5 treatment is a novel therapeutic strategy which may offer many clinical benefits to an asthmatic patients, selected on the basis of recurrent asthmatic exacerbation due to eosinophilic airway inflammation. Certainly, such a strategy is not an option for patients suffering from moderate persistent asthma, particularly if they do not need frequent oral corticosteroid courses to obtain asthma control. On the other hand, patients who, despite receiving systemic glucocorticoids, had peripheral blood eosinophil count well above 150 cells/mcL and frequent asthma exacerbations would experience better control of asthma symptoms along with reduced exacerbation rates [23, 43, 44].

Furthermore, the glucocorticoid-sparing effect of anti-IL-5 therapy [44] may prevent the serious, often irreversible adverse effects of glucocorticoids.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Managing Severe Asthma: A Role for the Long-Acting Muscarinic Antagonist Tiotropium

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Severe asthma is associated with substantial morbidity and mortality. Therapies must be maximized to gain control of a patient's severe asthma; however, avoiding overtreatment is also important. The mainstays of asthma maintenance treatment are inhaled corticosteroids (ICS) and long-acting β_2-agonsits (LABAs), with the option of supplementary add-on treatments. New add-on treatments for severe asthma have emerged over the past two decades, including personalized biological therapies that are guided by a patient's asthma phenotype. In addition, the long-acting muscarinic antagonist tiotropium has been recommended as an add-on treatment for severe asthma. Phase III clinical trials have shown tiotropium in combination with ICS/LABA to be efficacious in patients with severe asthma. Further analyses of clinical trial data have indicated that there is no benefit in stratifying patients by phenotype to predict tiotropium efficacy. Furthermore, health economic studies suggest tiotropium to be a cost-effective treatment in patients with severe asthma. This review will present the evidence surrounding the role of tiotropium in severe asthma and will discuss the use of tiotropium add-on therapy before personalized medicine strategies in the stepwise process of gaining asthma control.

1. Introduction

For the estimated 358 million patients worldwide who live with asthma, management of their disease has the overarching goal of gaining complete control and minimizing future risk [1]. Control is defined as the suppression of asthma symptoms and exacerbations, the removal of rescue medication need, restoration of normal lung function, and the reversal of activity limitation due to asthma [2]. Moreover, control of asthma includes reductions in the future risk of exacerbations, lung function decline, worsening control, and medication increase. In fact, current control has been shown to predict future risk of exacerbations, instability, and future lung function decline [3, 4]. However, asthma severity varies greatly between patients [5]. Accordingly, recommended treatment strategies also vary, with more aggressive treatment recommended for more severe asthma in order to gain control of the disease. Furthermore, the aim is for the patient to achieve asthma control whilst experiencing minimal treatment side effects [5]. This means patients should receive only the therapy required to achieve complete control and not unnecessary additional interventions.

Despite treatment in accordance with guidelines, including the use of inhaled corticosteroids (ICS) and/or long-acting β_2-agonists (LABA), a proportion of patients continue to have impaired control and experience the symptoms of asthma [2, 5, 6]. For these uncontrolled patients, treatment may be increased in the form of dosage or employing additional therapies [5]. Extrinsic factors such as low adherence to therapy, a reluctance of patients and carers to use corticosteroids, insufficient patient and clinician disease education, comorbidities, and environmental risk factors (for example, allergens and tobacco smoke) also contribute to uncontrolled asthma [5, 7–9]. Poor management of these extrinsic factors defines difficult-to-treat asthma [5].

The Global Initiative for Asthma (GINA) 2018 asthma management strategy follows a stepwise escalation in therapy so as to gain control of a patient's asthma (Figure 1) [5]. GINA 2018 defines severe asthma as asthma that remains uncontrolled despite, or that is only controlled by, Steps

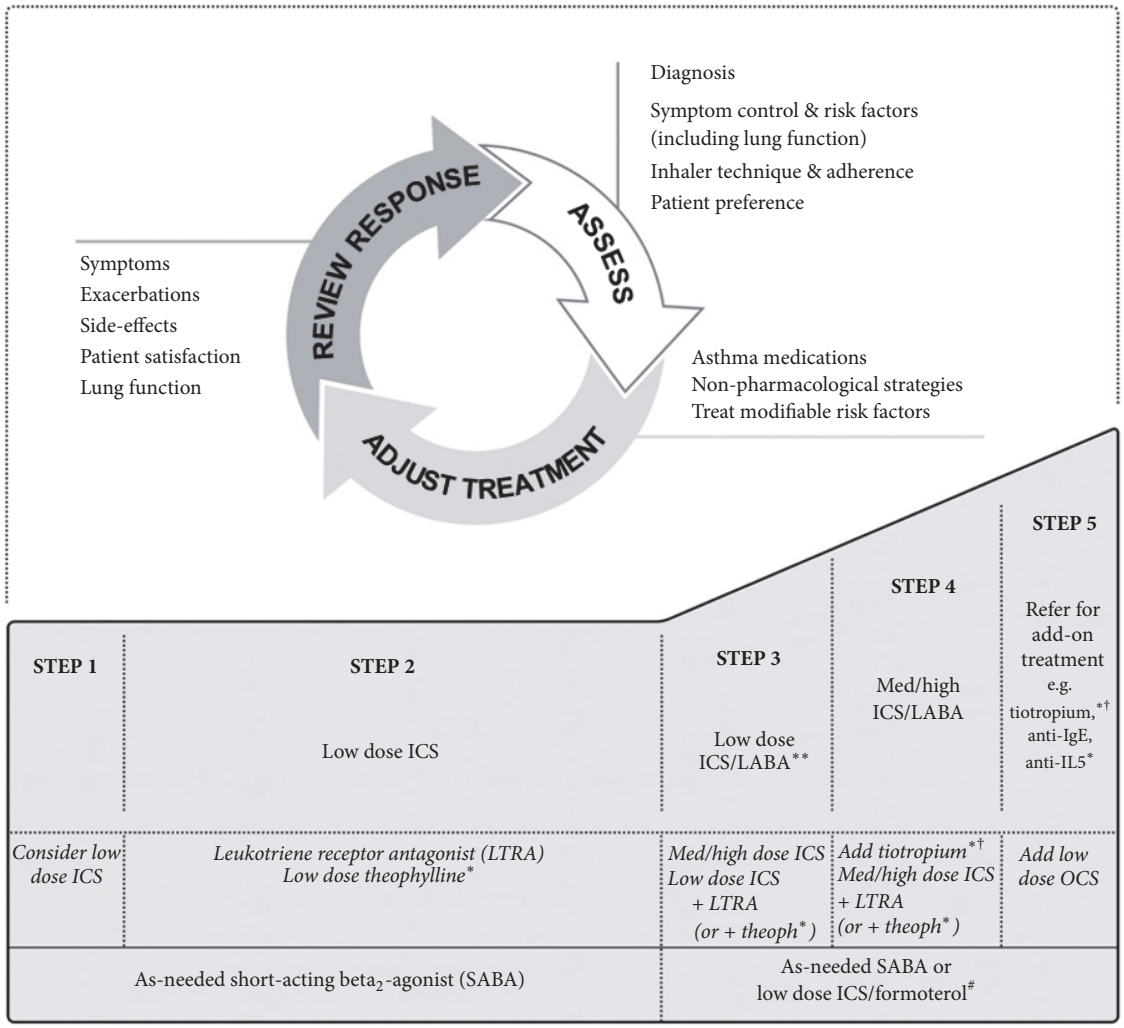

Figure 1: Stepwise asthma management in adults, adolescents, and children aged 6–11 years. *Notes*. *Not for children aged 12 years; **for children aged 6–11 years (preferred Step 3 treatment medium-dose ICS); #for patients prescribed BDP/formoterol or BUD/formoterol maintenance and reliever therapy; †tiotropium by mist inhaler is an add-on treatment for patients aged ≥12 years with a history of exacerbations. Copyright ©2018 Global Initiative for Asthma. Reproduced with permission from. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2018. *Abbreviations*. BDP, beclomethasone dipropionate; BUD, budesonide; ICS, inhaled corticosteroid; LABA, long-acting β_2-agonist.

4–5 of treatment; these steps are comprised of two or more controllers, usually medium-to-high dose ICS/LABA, plus as-needed reliever medication (Figure 1, Steps 4–5) [5]. The morbidity and mortality of patients with severe asthma are substantial: 26% of patients are not working due to their disease, and an estimated 39% of asthma deaths are of patients with severe asthma [10, 11]. Severe refractory asthma—a subset of severe asthma cases, defined as uncontrolled asthma despite management of extrinsic factors—represents an estimated 3.6% of asthma cases, equating to 12.9 million cases worldwide [1, 6].

Over the past two decades, the available spectrum of add-on drugs approved for use in asthma has broadened. These include small-molecule leukotriene modifiers and monoclonal antibodies, both of which target the immune component of asthma, as well as bronchodilators [12]. In addition to the development of new drugs, research into the pathology of asthma has revealed the disease to be a complex and heterogeneous disease. Patients can now be stratified into different subtypes of asthma, such as allergic or type 2-high (T2-high) phenotypes [13]. This involves the measurement of biomarkers such as blood eosinophil count, blood immunoglobulin E (IgE) levels, and the fraction of exhaled nitric oxide [13]. Personalized therapy plans can then be tailored to each patient in accordance with their subtype of the disease (see other reviews in this special issue). Clinical guidelines reflect these developments, with the GINA 2018 report suggesting patients with severe asthma who remain uncontrolled on ICS/LABA may be phenotyped and treated with appropriate biological therapies [5]. However, phenotyping patients may be time-consuming, and phenotypes may not be stable over time [14, 15]. Furthermore, personalized

therapies are expensive, primarily constituting monoclonal antibody-based drugs, and are not widely available for patients under the age of 18.

Long-acting muscarinic antagonists (LAMAs) are a class of bronchodilators with a mechanism of action that is distinct from LABAs. Inhibition of the muscarinic receptors of the bronchioles causes relaxation of the smooth muscle; furthermore, inhibition has been shown to reduce inflammation and asthma-related airways remodelling in preclinical asthma models [16–19]. Tiotropium is the first LAMA add-on therapy approved for use in asthma. This review will present the evidence surrounding the role of tiotropium add-on therapy in severe asthma management and discuss how it may be a broadly effective and economical therapy for use before personalized medicine strategies.

2. Where Do LAMAs Fit into Severe Asthma Management?

As described in the GINA 2018 report, achieving asthma control requires a cyclical approach to patient management (Figure 1) [5]. Patients are initially assessed for asthma control: if their disease is uncontrolled, new treatment may be provided; if the patient has had 3 months of asthma control, a reduction in treatment may be considered [5]. Reviewing the impact of changes in treatment on asthma control allows patients and clinicians to make a judgement on whether treatment should be adjusted, thereby restarting the assessment cycle. However, this process relies upon the clinician and the patient ensuring all symptoms are accurately reported and assessed, appropriate treatments are trialled, and treatments are properly adhered to. In fact, an estimated 79.5% of uncontrolled asthma cases are thought to be due to failure to adhere to asthma medications and poor inhaler technique, rather than truly medication-resistant disease [6].

Tiotropium is a new addition to the range of treatments that may be trialled in asthma patients experiencing suboptimal asthma control. First approved for use in asthma in 2014, tiotropium is licenced for use as a once-daily maintenance add-on therapy in patients aged 6 years and older in the US and EU and in patients aged 15 years and older in Japan [20–22]. GINA recommends tiotropium for use in severe asthma (Steps 4 and 5) as an add-on treatment to medium-to-high dose ICS/LABA in patients aged ≥12 years (Figure 1) [5]. Specifically, GINA placed tiotropium beginning with Step 4 treatment and before biologics or oral corticosteroids (OCS) (Figure 1). Similarly, German, Spanish, and UK asthma guidelines recommend tiotropium add-on use in patients with severe asthma as an option for add-on therapy when high-dose ICS/LABA therapies fail to gain asthma control; however, this recommendation is for adults only [2, 23, 24].

2.1. Clinical Studies Investigating Tiotropium in Patients with Severe Asthma. Current guidelines have based their recommendations on evidence from Phase III clinical studies investigating the use of tiotropium add-on therapy in severe asthma (Table 1). In the two replicate Phase III PrimoTinA-asthma trials, 912 adult patients with symptomatic severe asthma received either tiotropium 5 μg or placebo, delivered by the Respimat Soft Mist inhaler, as add-on maintenance therapy to at least ICS/LABA [25]. The first co-primary endpoint—change from baseline (response) in peak forced expiratory volume in 1 second (FEV_1) within 3 hours after dose ($FEV_{1(0-3h)}$) at Week 24—was significantly greater in patients receiving tiotropium add-on compared with placebo (86–154 mL, P<0.05). The second co-primary endpoint—trough FEV_1 response at Week 24—was significantly greater in the tiotropium add-on arm compared with the placebo arm (88–111 mL, P<0.05). The third co-primary endpoint—the time to the first severe asthma exacerbation (an exacerbation was defined as deterioration of asthma requiring OCS for ≥3 days)—was increased with tiotropium by 56 days compared with placebo (282 days versus 226 days). This corresponded to a reduction in risk of exacerbation of 21% with tiotropium compared with placebo (odds ratio [OR] 0.79, P=0.03), with the total number of exacerbations per patient-year being 0.53 and 0.66 for patients receiving tiotropium or placebo, respectively. This result shows that tiotropium can reduce the number of patients with severe asthma requiring OCS. This effect was despite inclusion criteria for the trials where patients were only required to have had a minimum of one exacerbation in the past year. Therefore, in contrast to recent trials for biologics [26–29], patients with a subtype of asthma that was highly prone to exacerbation were not specifically selected. Nonetheless, an increased median time to first asthma worsening—a secondary endpoint defined as either a progressive increase in symptoms or a decline of ≥30% in morning peak expiratory flow at screening for 2 consecutive days—was also found (hazard ratio 0.69, P<0.001). In line with this, the PrimoTinA-asthma trials showed some indication that tiotropium provides improvements in asthma symptom control, a secondary endpoint for the trials. Trial 2 of PrimoTinA-asthma showed a significant improvement in patients' seven-question Asthma Control Questionnaire (ACQ-7) score (–0.2, P=0.003), even though the effect in trial 1 did not reach statistical significance (–0.13, P=0.06). In a post hoc pooled analysis of both trials, ACQ-7 responder rate (a responder was defined by having a decrease in ACQ-7 score from baseline ≥0.5, which is considered the minimum clinically important difference) was significantly improved at Week 24 (OR 1.32, P=0.04) and at Week 48 (OR 1.68, P<0.001) [30]. Taken together, the lung function improvements, exacerbation and asthma worsening reductions, and symptom reductions reported in the PrimoTinA-asthma trials show that tiotropium has utility in gaining asthma control for adult patients with severe asthma.

Efficacious add-on therapies for paediatric patients with severe asthma are of particular interest as they may reduce the need to increase ICS dose, which is associated with a reduction in growth [31, 32]. A Phase III trial in adult patients has shown tiotropium to be superior to doubling ICS dose in terms of the proportion of days with asthma control, improvement in lung function, and improvements in asthma symptoms [33]. Phase III trials investigating tiotropium efficacy and tolerability in the paediatric setting have shown positive results across a range of severities, including symptomatic severe asthma. In the

Table 1: Phase III trials investigating tiotropium in adults, adolescents, and children with severe asthma.

Trial(s)	ClinicalTrials.gov number(s)	Background treatment	Age, years	Trial duration, weeks	Week of primary endpoint reporting	Patients, n	Tiotropium 5 μg versus placebo[a], mL	Tiotropium 2.5 μg versus placebo[a], mL
PrimoTinA-asthma [25]	NCT00772538 NCT00776984	High-dose ICS + LABA	18–75	48	24	912	**Peak $FEV_{1(0-3h)}$ response:** 86, P<0.05 154, P<0.001 **Trough FEV_1 response:** 88, P<0.01 111, P<0.001 **ACQ response:** −0.13, P=0.06 −0.20, P=0.003 **ACQ responder rate:** NR	N/A
PensieTinA-asthma [35]	NCT01277523	High-dose ICS + ≥1 controllers[b] or Medium-dose ICS + ≥2 controllers[b]	12–17	12	12	392	**Peak $FEV_{1(0-3h)}$ response:** 90, P=0.104 **Trough FEV_1 response:** 54, P=0.361 **ACQ-7 response:** 0.036, P=NR **ACQ-7 responder rate:** NR, P=0.952	**Peak $FEV_{1(0-3h)}$ response:** 111, P=0.046 **Trough FEV_1 response:** 115, P=0.051 **ACQ-7 response:** 0.058, P=NR **ACQ-7 responder rate:** NR, P=0.802
VivaTinA-asthma [34]	NCT01634152	High-dose ICS + ≥1 controllers[b] or Medium-dose ICS + ≥2 controllers[b]	6–11	12	12	400	**Peak $FEV_{1(0-3h)}$ response:** 139, P<0.001 **Trough FEV_1 response:** 87, P=0.01 **ACQ-IA response:** −0.08, P=0.32 **ACQ-IA responder rate:** 80.8% vs. 76.9%, P=NR	**Peak $FEV_{1(0-3h)}$ response:** 35, P=0.27 **Trough FEV_1 response:** 18, P=0.59 **ACQ-IA response:** 0.02, P=0.80 **ACQ-IA responder rate:** 79.4% vs. 76.9%, P=NR

[a]At week of primary endpoint reporting; [b]e.g., LABA and/or leukotriene receptor antagonist and/or sustained-release theophylline. ACQ-7, seven-question Asthma Control Questionnaire; ACQ-IA, interviewer-administered version of the Asthma Control Questionnaire; FEV_1, forced expiratory volume in 1s; $FEV_{1(0-3h)}$, FEV_1 within 3 hours after dose; LABA, long-acting β_2-agonsit; N/A, not applicable; NR, not reported; NS, not significant.

VivaTinA-asthma trial, a 12-week study involving 400 children (aged 6–11 years) with symptomatic severe asthma receiving ICS plus ≥1 controller therapy as maintenance treatment, tiotropium 5 μg add-on improved peak $FEV_{1(0-3h)}$ response at Week 12 versus placebo add-on (adjusted mean difference: 139 mL, 95% confidence interval [CI] 75–203, P<0.001) [34]. Tiotropium has also been evaluated in 392 adolescent patients (aged 12–17 years) with symptomatic severe asthma receiving ICS plus ≥1 controller therapy in the PensieTinA-asthma trial [35]. This 12-week parallel assignment trial did not meet the primary endpoint, with tiotropium 5 μg add-on therapy only numerically improving peak $FEV_{1(0-3h)}$ response versus placebo add-on at Week 12 (90 mL, 95% CI −19 to 198, P=0.104). However, the lower dose of tiotropium 2.5 μg did show nominally significant improvement in peak $FEV_{1(0-3h)}$ response versus placebo add-on at Week 12 (111 mL, 95% CI 2–220, P=0.046). Both the VivaTinA-asthma and PensieTinA-asthma trials investigated the effect of tiotropium add-on treatment on symptoms via the interviewer-administered version of the Asthma Control Questionnaire (ACQ-IA) and the ACQ-7, respectively. Both trials reported no significant difference in responder rate between tiotropium add-on and placebo [34, 35]; however, there was improvement in ACQ-AI or ACQ-7 score in all treatment arms, including the placebo groups, possibly due to improved background medication compliance in the trial setting [36, 37]. This strong placebo effect makes interpretation of these trial results challenging. Importantly, both the PensieTinA-asthma and VivaTinA-asthma trials found that tiotropium add-on therapy was well tolerated, with comparable or lower numbers of patients reporting adverse events compared with placebo.

A recent meta-analysis of the PensieTinA-asthma and VivaTinA-asthma trials, pooling data from 792 paediatric patients, found that peak $FEV_{1(0-3h)}$ response at Week 12 was significantly improved in patients receiving tiotropium add-on versus placebo (tiotropium 5 μg: 117 mL, P=0.0005; tiotropium 2.5 μg: 74 mL, P=0.0273) [38]. Similarly, trough FEV_1 response was significantly greater with tiotropium 5 μg add-on versus placebo (tiotropium 5 μg: 71 mL, P=0.0395; tiotropium 2.5 μg: 64 mL, P=0.0617). Patients receiving tiotropium add-on versus placebo were found to have significantly greater forced expiratory flow at 25–75% of forced vital capacity (FVC) ($FEF_{(25-75\%)}$) response (tiotropium 5 μg: 296 mL/sec, P<0.0001; tiotropium 2.5 μg: 211 mL/sec, P=0.0012) and trough FEV_1/FVC ratio (tiotropium 5 μg: 1.921%, P=0.0040; tiotropium 2.5 μg: 1.930%, P=0.0038).

Asthma exacerbations and worsening were not primary endpoints in either the PensieTinA- or VivaTinA-Asthma trials, and thus the trials were not powered toward detecting an effect. In particular, the trial lengths of 12 weeks, agreed upon with the regulatory bodies, were insufficient to detect significant effects on exacerbations in the single trials. Nevertheless, a meta-analysis pooling data from the PensieTinA- and VivaTinA-asthma trials has indicated tiotropium may have some activity in reducing asthma worsening in the paediatric severe asthma setting [39]. Time to first asthma worsening in this pooled analysis of 792 patients was significantly increased with tiotropium compared with placebo (tiotropium 2.5 μg: P=0.009; tiotropium 5 μg: P=0.029).

These data underline the efficacy of tiotropium in severe paediatric asthma and, in line with this, tiotropium 5 μg add-on therapy has recently been approved in the EU for use in children aged 6 years and older with symptomatic asthma [21]. Furthermore, the data from these paediatric trials support those from the PrimoTinA-asthma trials in showing that tiotropium is an efficacious therapy for the treatment of severe asthma, with a significant effect on improving lung function across a broad range of ages. However, this conclusion must be applied within the context of the patient populations studied, namely, adult patients with persistent symptoms and reversible airways obstruction despite receiving high-dose ICS/LABA and paediatric patients aged 6–17 years with persistent symptoms and reversible airways obstruction despite receiving high-dose ICS plus additional controller therapies.

3. The Role of LAMAs in Personalized Therapy

Stratifying patients for personalized treatment, especially those with severe asthma, is being discussed as the treatment approach of choice (see other reviews in this special issue). This raises the question: should a personalized treatment approach be applied to tiotropium therapy?

To address this question in an adult patient population with severe asthma, Kerstjens and colleagues performed post hoc analyses using pooled data from the PrimoTinA-asthma trials to determine whether baseline characteristics influenced tiotropium efficacy [30]. The analysis focused on the endpoints peak $FEV_{1(0-3h)}$ response and trough FEV_1 response at Week 24 and time to first asthma exacerbation and first asthma worsening over 48 weeks. None of these endpoints were significantly influenced by any baseline characteristic investigated, including sex, age, body mass index, disease duration, age of asthma onset, or smoking status, thus supporting the efficacy of tiotropium across a broad range of patients with severe asthma.

Inflammation, both allergic and nonallergic, is a significant feature of asthma. Elevated eosinophilic inflammation and elevated IgE levels, as well as the release of cytokines such as interleukin-5 (IL-5) and interleukin-13, define the T2-high asthma phenotype [40]. The T2-high phenotype is used in clinical practice to stratify patients for biological therapies such as anti-IgE and anti-IL-5 antibodies [41]. Casale and colleagues recently investigated whether the efficacy of tiotropium was influenced by the T2 phenotype status [42]. Their post hoc analysis used data from four large Phase III trials: PrimoTinA-asthma (two replicate trials involving 912 adult patients with symptomatic severe asthma where patients were receiving at least ICS/LABA maintenance therapy) and MezzoTinA-asthma (two replicate trials involving 2100 adult patients with symptomatic moderate asthma where patients were receiving at least ICS maintenance therapy). The analysis found that tiotropium improved lung function versus placebo in all trials regardless of baseline phenotype. Analysis of the PrimoTinA-asthma (severe asthma) trials revealed that tiotropium improved peak $FEV_{1(0-3h)}$ by

102 mL (P<0.01) and 148 mL (P<0.001) versus placebo in patients with both high (>430 μg/L) and low (≤430 μg/L) baseline serum IgE, respectively. Trough FEV_1 was improved by 89 mL (P<0.01) and 127 mL (P<0.001) in patients with both high and low serum IgE levels, respectively. Baseline serum IgE levels also had no significant effect on the risk of exacerbation for tiotropium versus placebo (interaction P=0.17) [30]. The authors reported similar improvements in peak $FEV_{1(0–3h)}$ response and trough FEV_1 response for all patients, irrespective of whether they were categorized as having allergic asthma by clinician judgement at baseline. Casale and colleagues also modelled the treatment effect of tiotropium over continuous ranges of phenotype biomarkers [42]. Their analysis found improvements in peak $FEV_{1(0–3h)}$ response and trough FEV_1 response in patients across a broad range of serum IgE levels and blood eosinophil counts at baseline. In addition to lung function improvements, the analysis indicated that asthma symptoms measured by the ACQ-7 score and the risk of asthma worsening were consistently improved with tiotropium therapy in patients with severe asthma across a range of serum IgE levels. This analysis suggests that there is no benefit in determining T2 phenotype status for the selection of patients with severe asthma who will benefit from tiotropium therapy.

A similar post hoc analysis has been conducted using pooled data from clinical trials involving paediatric patients (aged 6–17 years) with moderate or severe asthma receiving placebo or tiotropium add-on therapy [43]. As with Casale et al., modelling of lung function endpoints across a continuum of baseline blood eosinophil counts and serum IgE levels was performed. The study found that peak $FEV_{1(0–3h)}$ response, trough FEV_1 response, FEV_1/FVC ratio, $FEF_{25–75\%}$ response, and in-clinic trough (evening) peak expiratory flow response improved with tiotropium therapy regardless of eosinophil blood count or IgE serum levels.

These findings in adult and paediatric patients, across a range of baseline characteristics, are perhaps to be expected because, as a bronchodilator, tiotropium should be beneficial in any patient with reversible airway obstruction. However, the results do provide important evidence that tiotropium is efficacious independent of disease subtype, negating the need for patient stratification. As such, tiotropium may be ideally placed as a therapy to be trialled in patients with uncontrolled severe asthma before undergoing phenotyping tests and pursuing personalized biological therapies. An important consideration is that we are unable to determine from current studies the extent to which tiotropium add-on therapy could reduce the number of patients requiring biologic treatment, although such data would be of great interest. However, the evidence presented would imply that a proportion of patients would gain benefit, and that this is irrespective of T2 status and therefore would not require prior phenotyping of patients.

4. LAMAs: A Cost-Effective Therapy for Severe Asthma?

An important consideration for biological therapy is cost. These therapies come with a significant economic burden for healthcare providers; for example, the estimated cost for the anti-IgE omalizumab and the anti-IL-5 mepolizumab monoclonal antibodies is $437 and $625 per patient per week, respectively [44, 45]. It is therefore prudent to tailor treatment strategies in such a way that the only patients to receive these expensive biological therapies are those that will benefit from them. As treatment is escalated for uncontrolled asthma, patients should trial each therapy in a systematic manner, as recommended in the 2018 GINA report [5]. As discussed above, tiotropium is an efficacious LAMA for patients with severe asthma irrespective of various phenotype characteristics. Tiotropium is therefore an obvious choice to be trialled during treatment step-up for patients with uncontrolled severe asthma, and the guidelines reflect this [2, 5].

Analysis of the cost-effectiveness of tiotropium in terms of improving asthma control and preventing exacerbations for patients with uncontrolled severe asthma has been conducted in the context of the UK healthcare system [46, 47]. Using 2012 prices, the authors reported the cost of tiotropium per patient per week to be £8.28, with lifetime cost over standard care to be £5389. Guidelines by the UK regulator, the National Institute for Health and Care Excellence, stipulate that an intervention must have a maximum threshold of £30,000 per quality-adjusted life year (QALY) in order to be classed as cost-effective. Analysis of tiotropium benefit revealed the addition of 0.19 QALYs over standard care, giving tiotropium a cost-effectiveness of £28,383 per QALY in the model. The authors therefore concluded this was a cost-effective intervention.

Recently, a study investigated the cost effectiveness of tiotropium in the US healthcare setting in patients with uncontrolled severe asthma [48]. The study used pricing data adjusted to the 2013 US consumer price index, reporting tiotropium cost per patient per week to be $13. The model estimated the lifetime cost of tiotropium therapy to be $3103 more than standard care. Furthermore, the authors reported that tiotropium add-on therapy added 0.09 QALYs over standard care. The cost-effectiveness of tiotropium add-on therapy in the analysis was $34,478 per QALY compared with standard care. The authors concluded that tiotropium was below a willingness-to-pay threshold of $50,000 per QALY and is therefore not a cost-effective treatment. The authors also compared tiotropium with omalizumab therapy in their cost-effectiveness model. Based on a price per patient per week of $437, the estimated lifetime cost of omalizumab compared with standard care or tiotropium was $179,415 and $176,312, respectively. Omalizumab added 0.38 QALYs over standard care and 0.29 QALYs over tiotropium. However, the high cost of omalizumab therapy meant cost-effectiveness was found to be $463,605 per QALY when compared with standard care and $593,643 per QALY when compared with tiotropium. The authors therefore concluded that tiotropium add-on therapy was more cost-effective than omalizumab and a cost-effective option compared with standard treatment.

Together, these studies suggest tiotropium is a relatively inexpensive and cost-effective therapy when stepping up treatment for patients with severe asthma. However,

this cost-effectiveness calculation is only applicable in a scenario where the use of tiotropium in patients with severe uncontrolled asthma results in sufficient quality of life improvements such that a step-up to a personalized biologic treatment is negated. Confirmatory studies are required to demonstrate such a biologic-sparing cost-benefit advantage for tiotropium. These would provide a better measure of the cost-effectiveness of tiotropium as a step-up treatment positioned between ICS/LABA and biologics.

5. Conclusions

An important aspect of severe asthma management is the use of add-on treatment to gain control of a patient's disease. This process should be stepwise and continuously reassessed, with appropriate therapies trialled to ensure patients receive the optimum treatment level required to control their asthma.

There is a substantial volume of data indicating that the LAMA tiotropium is an efficacious add-on treatment for use in patients whose severe asthma remains uncontrolled despite combination therapy with ICS and additional controller therapies. The evidence shows improvements in lung function measures, as well as an indication of reductions in risk of and time to asthma exacerbation or worsening and symptom reduction. Importantly, post hoc analyses have suggested that tiotropium is broadly efficacious irrespective of asthma phenotype, meaning tiotropium may be utilized without additional characterization of a patient's asthma. Since better daily control and higher lung function act in a protective manner against loss of control/exacerbation in the long run, tiotropium might help to stabilize patients. As such, the GINA 2018 report recommends tiotropium as an add-on therapy to ICS/LABA before stepping up to biologics for patients with uncontrolled asthma.

Tiotropium is cost-effective and substantially less expensive than biological therapies. Hence, it seems that tiotropium is ideally placed as an add-on therapy that can be trialled in patients prior to additional phenotype-guided therapies or increased ICS dose. This is particularly important in children and adolescents, in whom high-dose ICS is linked to impaired growth. There is a need to conduct further studies in this area to confirm that treatment with tiotropium can reduce the need to step up treatment to phenotype-guided therapies and to calculate the possible cost savings associated with this, in patients with severe asthma. Despite this, escalation to phenotype-specific personalized biological therapies may still be required when asthma remains uncontrolled despite active management and comprehensive trialling of add-on therapies.

Disclosure

The author takes full responsibility for the scope, direction, content of, and editorial decisions relating to the manuscript, was involved at all stages of development, and has approved the submitted manuscript.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this article.

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Review Article

Omalizumab for Severe Asthma: Beyond Allergic Asthma

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Different subsets of asthma patients may be recognized according to the exposure trigger and the frequency and severity of clinical signs and symptoms. Regarding the exposure trigger, generally asthma can be classified as allergic (or atopic) and nonallergic (or nonatopic). Allergic and nonallergic asthma are distinguished by the presence or absence of clinical allergic reaction and in vitro IgE response to specific aeroallergens. The mechanisms of allergic asthma have been extensively studied with major advances in the last two decades. Nonallergic asthma is characterized by its apparent independence from allergen exposure and sensitization and a higher degree of severity, but little is known regarding the underlying mechanisms. Clinically, allergic and nonallergic asthma are virtually indistinguishable in exacerbations, although exacerbation following allergen exposure is typical of allergic asthma. Although they both show several distinct clinical phenotypes and different biomarkers, there are no ideal biomarkers to stratify asthma phenotypes and guide therapy in clinical practice. Nevertheless, some biomarkers may be helpful to select subsets of atopic patients which might benefit from biologic agents, such as omalizumab. Patients with severe asthma, uncontrolled besides optimal treatment, notwithstanding nonatopic, may also benefit from omalizumab therapy, although currently there are no randomized double-blind placebo controlled clinical trials to support this suggestion. However, omalizumab discontinuation according to each patient's response to therapy and pharmacoeconomical analysis are questions that remain to be answered.

1. Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity, together with variable expiratory airflow limitation [1].

The prevalence of asthma, one of the most common chronic diseases in the world [2, 3], has increased during the 1970s and 1980s. Epidemiologic studies from the 90s suggested that the prevalence of asthma was around 7.7% in the United States (US)—over 22 million people—and lethality rate was estimated at 5.2 per 100,000 asthmatic patients per year. Worldwide, 200–300 million people suffer from asthma [1–3], and severe asthma comprises 5-10% of all asthmatic patients [4]. In Portugal, the prevalence of asthma is estimated to be of 6.8% [5], affecting around 1 million people. Of these, only 57% have controlled disease, which means that around 300,000 Portuguese asthmatics need a better intervention to control their disease.

The number of hospitalizations due to asthma was 2,728 in 2016, from a total of 262,229 asthmatic patients registered

in the Portuguese National Health Service. The standardized mortality rate was, in 2015, of 4.0/100,000 inhabitants for patients above 65 years of age, and of 0.1/100,000 inhabitants for patients below 65 years of age. Nevertheless, and according to the latest Organisation for Economic Co-operation and Development (OECD) report, Portugal is among the countries with less mortality and the country with less hospitalizations due to asthma [6].

The high prevalence of asthma, the impairment of quality of life, the absenteeism, and the large health resources needed to manage this disease makes the economic burden of asthma one of the highest among all chronic diseases. Asthma-related costs have been estimated at up to 2% of the economic cost of all diseases in developed countries [7]. A recent systematic review examined 68 papers on the economic burden of asthma between 1966 and 2008 and concluded that despite the availability of effective preventive therapies, the cost of asthma treatment has increased significantly over the last few decades [8]. A study conducted in Portugal in 2010 concluded that asthma in adults poses a significant economic burden on the Portuguese healthcare system. Total costs amounted to a grand total of €386,197,211.25, with direct costs representing 93% or €359,093,559.82, 2.04% of the total Portuguese healthcare expense in 2010. The major costs were acute care usage (30.7%) and treatment (37.4%). A considerable portion of this burden might be eased by improving asthma control in patients, as uncontrolled patients' costs are more than double those of controlled asthma patients [9].

Severe asthma has a heterogeneous definition. The World Health Organization (WHO) suggests that severe asthma includes three groups: (1) untreated asthma; (2) incorrectly treated asthma (as a result of nonadherence, persistent triggers, or comorbidities); and (3) difficult-to-treat asthma. It is also important to distinguish between severe asthma, comprising patients requiring medium/high doses of inhaled corticosteroids in combination with LABA or other controller, and uncontrolled asthma, resulting from inappropriate therapy or persistent problems with adherence or comorbidities [1]. According to the British Guidelines for Asthma, difficult asthma is defined as that with persistent symptoms and/or frequent asthma attacks despite treatment with high-dose therapies or continuous or frequent use of oral steroids [10]. Untreated patients have been recently omitted in the 2014 revision document produced by the task force of the European Respiratory Society (ERS) and the American Thoracic Society (ATS) [11].

Regarding the exposure trigger, generally asthma can be classified as allergic (or atopic) and nonallergic (or nonatopic or intrinsic) asthma. Allergic and nonallergic asthma are distinguished by the presence or absence of clinical allergic reaction and in vitro IgE response to specific aeroallergens [12, 13]. The triggering of an inflammatory cascade mediated by Immunoglobulin E (IgE) mast cells' activation, with eosinophils and Th2 lymphocyte synthesis, mobilization, and activation in the airways with IL-4, IL-5, and IL-13 production, leads to bronchial constriction and mucus production with airways narrowing [14–21]. The mechanisms of allergic asthma have been extensively studied with major advances happening in the last two decades. Nonallergic asthma is characterized by its apparent independence from allergen exposure and sensitization, but also by a higher degree of severity [12, 13].

Of note, it is important to distinguish nonallergic asthma from aspirin exacerbated respiratory disease (AERD) which also has its own epidemiology, physiopathology, and clinical features: these patients often develop asthma symptoms years after developing rhinitis and nasal polyps due to increased production of cysteinyl–leukotrienes most probably as a result of a polymorphism of the cysteinyl–leukotriene synthase gene [22].

Whether these different clinical subsets of asthma are due to different etiopathogenesis or a different spectrum (or phenotype) of the same disease resulting from different underlying unrecognized mechanisms is still a matter of ongoing debate [15, 23].

This review was prepared and discussed by a group of specialists belonging to the Portuguese Network of Severe Asthma Specialists—REAG.

1.1. Allergic versus Nonallergic Asthma. There are similar clinical and physiopathological phenomena between allergic and nonallergic asthma: both can be triggered by exercise, inhaled irritants, or upper airway tract infection; both are associated with rhinitis and both can have higher total serum IgE, airways IgE, airways Th2 cells and Th2, and eosinophilic chemokines and cytokines. Recently, different studies have tried to find a common pathophysiological and immunobiological pattern between both forms of asthma. According to these studies, nonallergic patients may produce the same inflammatory mediators as allergic patients after local IgE production by T lymphocytes at the bronchial and lung mucosal surface where antigens are presented. This was demonstrated comparing bronchial biopsies samples of nonatopic asthma patients, atopic asthma patients, and nonasthmatic controls [12, 24–27].

Clinically, allergic and nonallergic asthma are virtually indistinguishable during exacerbations, since both lead to signs and symptoms of variable lower airways narrowing and obstruction, which is reversible, at least partially, with bronchodilators [14, 18, 20, 21, 28].

By definition, allergic asthma is clearly associated with allergenic triggering, positive skin prick test, and raised specific IgE (sIgE) [15, 23, 29]. On the other hand, nonallergic asthma is usually of late onset, shows no familial patterns and no genetic trends have been recognized [15, 23, 30], has a higher female prevalence, and tends to be of difficult control and with more severe relapses. A patient with asthma is diagnosed with nonallergic asthma if skin prick tests are negative and no circulating sIgE are found [14, 18, 20, 21, 28, 31, 32].

The relationship between allergic and nonallergic asthma prevalence is difficult to ascertain. In some studies, nonallergic asthma prevalence appears to be increasing more than allergic asthma [15]. According to the Swiss Sentinel Surveillance Network (SSSN), the consultations for asthma have decreased over time mainly due to a decrease of allergic asthma. Consultations for nonallergic asthma did not change significantly between 1999 and 2005 [33].

Table 1: Severe asthma phenotypes proposed by Campo *et al.* [42].

Clinical phenotypes	Characteristics
Asthma with frequent severe exacerbations	Frequent severe exacerbations with periods of relative stability between exacerbations
Asthma with fixed airflow obstruction	Irreversible persistent and progressive airflow obstruction
Corticosteroid-dependent asthma	Symptoms cannot be controlled, despite high doses of ICS, and patients require daily doses of OCS. Reducing the dose of OCS can often lead to clinical worsening and exacerbations
Inflammatory phenotypes	
Persistent severe eosinophilic asthma	Eosinophilia in bronchial biopsies and induced sputum despite high doses of ICS or OCS. Characterized by more symptoms, lower FEV_1 values, and more severe exacerbations than the non-eosinophilic subtype
Non-eosinophilic severe asthma with increased neutrophils	Eosinophils are either absent from the airway or suppressed by treatment despite the presence of several symptoms, with inflammation of the airway characterized by an increased percentage of neutrophils
Severe paucigranulocytic asthma	It does not involve inflammation by the classical cell types in the bronchial biopsy. Inflammation may be located in the distal airway, which is inaccessible for biopsy, or it may be due to a bronchiolitis-type disease. No thickening of the subepithelial basement membrane or signs of classic inflammation are observed. Other inflammation pathways and other cell types could also be activated

ICS: inhaled corticosteroids; OCS: oral corticosteroids.

The true prevalence of severe asthma among nonallergic patients compared to allergic asthma patients is uncertain. Most of the studies assume that severe disease is more prevalent among nonatopic asthma patients. There are conflicting data regarding prevalence trends of asthma and atopy over the last 10–15 years [33]. The proportion of asthmatics with severe disease and a negative skin prick test varies from 17 to 34% in the Severe Asthma Research Program (SARP) study [34] to 50% in the ENFUMOSA study [35]. In the ENFUMOSA study, a cross-sectional analysis, it was found that patients with severe asthma were less likely to be skin prick-positive and more likely to have high levels of neutrophils in sputum than patients with less severe asthma [35]. On the other hand, the U-BIOPRED cohort [36] reported a 76.6% incidence of atopy in severe asthma, including nonsmokers, smokers, and ex-smokers.

Although the prevalence and social and financial burdens of nonallergic asthma seem to be lower than in allergic asthma [19], from a clinical point of view, nonallergic asthma is a true challenge: these patients are usually the most difficult to diagnose, due to their specific epidemiologic features, and the most difficult to treat and control.

1.2. Phenotypes. There is a complex network of different mechanistic and clinical features which are likely linked by a common pattern of reversible respiratory distress associated to distal airways narrowing. In the last decades efforts have focused on the classification of different subsets of asthma patients according to its epidemiology, immunology, biomarkers, response to specific pharmacotherapies, and long-term prognosis. These are broadly called phenotypes: a set of clinical features of a specific genetic pattern in a specific environment. The main goal of the phenotype and endotype philosophy is the development of targeted and personalized pharmacological approaches. Phenotype definition is particularly important in patients with moderate to severe disease and who are not controlled with usual therapy. A detailed and systematic clinical history, including comorbidities, spirometry with bronchodilator test, a skin or blood test panel for sIgE to common regional airborne allergens, and a peripheral blood eosinophil count are very useful for establishing phenotypes. With this information, allergic and nonallergic asthma and eosinophilic or noneosinophilic asthma can be distinguished. This distinction has prognostic and therapeutic implications.

However, although the above-mentioned four phenotypes are considered to be the major ones, research on asthma phenotypes has increased exponentially in the last years and cluster analysis has identified several distinct clinical phenotypes of asthma [34, 37–39]. There is, nonetheless, a clear heterogeneity regarding asthma phenotypes. GINA considers five phenotypes [1] and Wenzel et al. proposed thirteen in 2006 [40]. However, in 2012, these thirteen phenotypes have been reduced to five, due to the evolution towards linking biology to phenotype, namely, at the molecular and genetic levels [41]. In 2013, Campo et al. [42] proposed 6 severe asthma phenotypes subdivided in clinical and inflammatory phenotypes—Table 1. Smoking is not a phenotype but a disease modifying factor with prognostic implications [42].

1.3. Biomarkers. Several biomarkers have been tested for diagnosis and prediction of clinical response to therapy in asthma, with the aim of achieving personalized therapy.

Severe asthma is usually characterized by a type 2 disease, associated with atopy and/or eosinophilic inflammation of the airways [43]. However, inflammation in severe asthma is not always characterized by the presence of eosinophils and cytokines of the high-Th2 endotype; in many cases, it may be low-Th2 neutrophilic or low-Th2 paucigranulocytic (type 1 disease) [42].

Currently there are several biomarkers for severe high-Th2 asthma, but there is a clear need to identify and select biomarkers of the low-Th2 endotypes. However, this is not an easy task, and several studies in severe asthmatics, such as the ENFUMOSA [35], TENOR [44], SARP [34], and, more recently, the U-BIOPRED [36], have shown a remarkable heterogeneity in the clinical presentation and in the underlying pathophysiological mechanisms of severe asthma.

1.3.1. High-Th2 Endotypes. Although heterogeneous, the classification of the high-Th2 endotypes is mainly based on sputum and systemic eosinophilia [45], and this is considered to be a relevant biomarker. These endotypes also show higher epithelial expression of total IgE [15, 44] and Th2 cytokines such as interleukines IL-4, IL-5, and IL-13 [15], two of which, IL-4 and IL13, directly contribute to IgE class switch, thereby increasing IgE [46]. Other known and established biomarkers of Th2 predominant asthma are exaled nitric oxide (FeNO) [47–50] and serum periostin [51]. In a recent study by Busse et al. [52], the authors defined high-Th2 as IgE ≥100 IU/ml, eosinophils count ≥ 300/μl, and FeNO ≥30 ppb. Currently, total IgE and serum eosinophils are used not only as disease biomarkers but also as variables on the treatment algorithm of a specific subgroup of severe asthmatic patients who are eligible for anti-IgE omalizumab [53] or anti-IL5 mepolizumab [54]. Indeed, an analysis of biomarkers of the EXTRA study [55] showed that combining biomarkers on the high-Th2 endotypes had therapeutic response implications: patients with severe atopic asthma with high IgE values and Th2 biomarkers (high blood eosinophils and periostin and high FeNO values) showed a better response to omalizumab therapy.

1.3.2. Low-Th2 Endotypes. Although high-Th2 asthma with atopy and eosinophilia is easy to identify, there is no accepted and consensual definition for the low-Th2 endotypes [56–58], which comprise around one-third of severe asthmatic patients [59].

Low-Th2 endotypes are currently identified in clinical practice as the absence of biomarkers of atopic asthma and/or eosinophilia. In the majority of cases, the low-Th2 endotypes are defined by the absence of Th2 inflammatory biomarkers and characterized as neutrophilic inflammation and, less frequently, by paucigranulocytic inflammation [42, 56].

Although there is no consensus regarding the percentage of sputum neutrophils that would define the neutrophilic asthma phenotype, some reports mention values between 40 and 70% [59].

Beyond the sputum leukocyte content, other specific biomarkers that are able to discriminate high-Th2 from low-Th2 are currently under investigation, but are still not applicable in clinical practice.

IL-8 is a cytokine associated with chemotaxis and neutrophilic degranulation and has been found to be elevated in the sputum of patients with severe resistant asthma [60–62]. CXCR1 and CXCR2 have been also found to be elevated in neutrophilic asthma [62]. Other potential biomarkers of neutrophilic asthma are myeloperoxidase [62] and neutrophilic elastase [61, 62] that can be assessed in sputum of this subgroup of severe asthmatics.

IL-17 is a biomarker of activation of the Th17 pathway, and correlations between the presence of IL-17 and the level of neutrophils in induced sputum and in circulation have been found in patients with severe asthma [62, 63].

There are currently no biomarkers for the subgroup of patients with paucigranulocytic asthma [62]. In this population of patients there is no predominant inflammatory type, and it is possible that other biomarkers of severe asthma, namely, biomarkers of airway remodelling such as osteopontin and angiopoietin, are relevant.

It is necessary to unravel the pathophysiological mechanisms of low-Th2 endotypes in order to identify future biomarkers of these subtypes of asthma [41, 56, 62].

Currently there are no accurate or precise biomarkers to stratify asthma phenotypes and guide therapy in clinical practice, as illustrated in Figure 1.

1.4. Effect of Interaction of Comorbidities. Uncontrolled allergic rhinitis, gastroesophageal reflux disease (GERD), obesity, vitamin D deficiency, noncompliance to therapy, and trigger exposure are among the most important effect modifiers of asthma. Of these, due to its prevalence, obesity is one of the most feared comorbidities in asthma patients.

Obese asthma patients show synergy among the two pathologies, i.e., the complexity of the disease is higher than the sum of the diseases, and this interaction worsens the prognosis. Obesity worsens preexisting asthma, through both biochemical and mechanical effects, and potentially impairs response to treatment, and obese patients are more likely to suffer from nonallergic asthma than nonobese patients [64, 65].

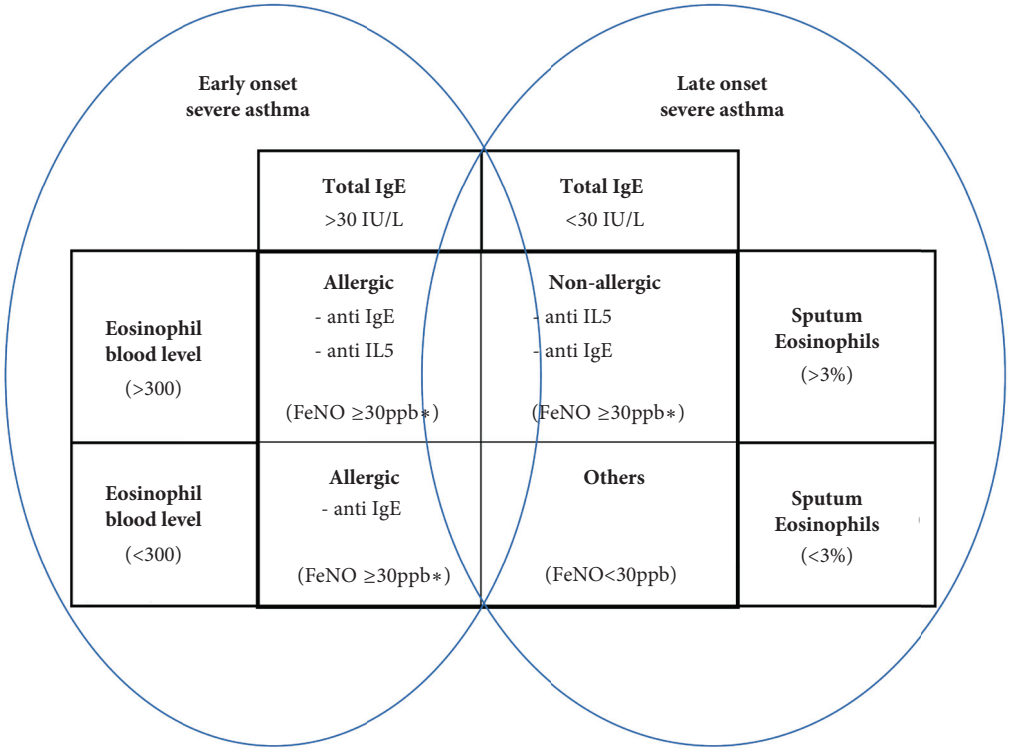
Even in obese asthmatic patients it seems to be possible to distinguish two different clinical courses based on age of onset and Th2 related biomarkers: early-onset asthma tends to have a more atopic disease, higher IgE, and greater bronchial hyperresponsiveness. These patients seem to have allergic asthma that is complicated by obesity. On the other hand, obese patients with late-onset asthma tend to have less atopy, bronchial hyperresponsiveness, and lower levels of Th2 inflammation. These patients have asthma that has developed in the setting of obesity [66].

2. Treatment Options for Severe Allergic and Nonallergic Asthma

The aim of therapy in asthma is achieving disease control. Disease control is considered by the British Thoracic Society [10] as

(i) no daytime symptoms

(ii) no night-time awakening due to asthma



Adapted from [69]

∗predictor of good response to anti-IgE treatment

Figure 1: Proposed biomarkers to stratify asthma by phenotypes are still not robust enough to guide therapy in clinical practice.

(iii) no need for rescue medication

(iv) no asthma attacks

(v) no limitations on activity including exercise

(vi) normal lung function (in practical terms FEV1 and/or PEF>80% predicted or best)

(vii) minimal side effects from medication.

The clinical management of nonallergic asthma is similar to that of allergic asthma. It comprises a combination of nonpharmacological approaches, namely, trigger avoidance and control of comorbidities and pharmacological approaches [1, 10, 67]. Pharmacological approach initiates with ICS as the mainstay of therapy with the addition of LABA if this is insufficient to control symptoms [1, 10, 67]. Additional add-on therapy to ICS and LABA according to disease control includes increasing doses of ICS or add-on LAMA, LTRA, or theophylline [1, 10]. Almost 90% of asthma patients can generally be controlled with ICS and LABA. Of the remaining 10%, between 17% and 50% are nonallergic asthma according to the SARP and ENFUMOSA studies [34, 35]. The U-BIOPRED study reported a 30% incidence of nonatopy in the asthma groups [36].

The presence of comorbidities should prompt the initiation of nonpharmacological and pharmacological strategies towards comorbidities, namely, obesity and GERD.

With the breakthrough of monoclonal antibodies (mAbs) therapies on the verge of the 21st century new pharmacological approaches have been developed and tested in these patients [24, 68]. Therapy with mAbs is a specific subset of immunotherapy using passive immunity in which preformed antibodies against a target antigen are injected into the body. MAbs can efficiently target an antigen blocking or initiating a biochemical cascade event and through this mechanism achieve a clinical response [24, 68]. This implies a much higher linkage between pathophysiology, clinical and pharmacotherapy to select the subset of patients who will benefit the most from biological therapy, which revisits phenotypes, immunobiology and endotypes.

2.1. Treatment Options in Severe Allergic Asthma. Sputum analysis and FeNO are very useful in predicting Th2 asthma phenotype, even if no eosinophilia is present. This is of utmost importance to therapeutic strategy definition: allergic asthma with elevated eosinophils and FeNO is more likely to respond to ICS [16] and omalizumab [55]. Allergic Th2 phenotype poorly controlled asthmatic patients should be considered good candidates for omalizumab therapy after add-on ICS/LABA/leukotriene/theophylline therapy [69–71].

Omalizumab is a monoclonal antibody designed to bind and inactivate IgE and was approved by EMA in 2009. For patients ≥6 years old omalizumab is indicated as add-on therapy to improve asthma control in patients with severe

persistent allergic asthma who have a positive skin test or in vitro reactivity to a perennial aeroallergen and frequent daytime symptoms or night-time awakenings and who have had multiple documented severe asthma exacerbations despite daily high-dose inhaled corticosteroids, plus a long-acting inhaled beta2-agonist. For patients ≥12 years of age a reduced lung function (FEV_1 <80%) is also required [72].

Omalizumab blocks free serum IgE and limits its binding to the FcεRI receptor on the surface of mast cells and basophils. This blockade leads to a reduction in the specific inflammatory response induced by activation of effector cells during the encounter with the allergen [73].

Omalizumab has been also demonstrated to reduce the expression of FcεRI on the surface of circulating mast cells and basophils [74, 75] which results in a decrease in the release of mediators induced by allergenic stimuli *in vitro* and *in vivo* [74, 76, 77]. Omalizumab also seems to intervene in the regulation of the number of circulating basophils which decreases in the treated child [78].

Beyond the anti-IgE mechanism centered on basophils and mast cells, several recent experimental data and clinical observations show that the mechanism of action of omalizumab is more complex than just blocking the allergic response, some of which are mentioned below.

Several studies have shown a decrease in the number of circulating eosinophils and bronchial tissue eosinophils in asthmatics treated with omalizumab [79–82]. Patients with steroid-resistant asthma have been shown to have higher levels of eosinophils, and in these cases omalizumab is a very effective treatment, reducing circulating eosinophils [83]. A proapoptotic effect of omalizumab on eosinophils may contribute to this decrease [84]. Moreover, a study exploring the potential of three biomarkers of Th2-driven inflammation (FeNO, peripheral blood eosinophils, and serum periostin) to predict response to treatment to omalizumab in patients with severe allergic asthma concluded that patients in the high-biomarker subgroup showed a significant decrease in the percentage of exacerbations compared to the low-biomarker subgroup, suggesting that these patients may achieve greater benefit from omalizumab therapy. However, the benefit of such a predictive biomarker of efficacy of omalizumab therapy is currently not established [55].

In a recent study of 673 patients, high levels of periostin and NO exhaled before treatment with omalizumab were associated with a significant decrease in the number of exacerbations [55]. Omalizumab appears to be targeting this Th2 inflammation and a decrease in exhaled NO after treatment has been found in various studies [85]. High levels of these markers prior to initiation of omalizumab have been proposed as biomarkers that predict efficacy with this therapy [55].

Various *in vitro*, *ex vivo*, and/or *in vivo* studies from blood samples, bronchial biopsies, or exhaled air condensates have shown mainly a decrease in the cytokines involved in the recruitment, activation, and survival of eosinophils and IL-5, IL-13, IL-4, IL-8, GM-CSF, eotaxin, RANTES, and the Th2 orientation of the immune response. IFN-γ, an anti-inflammatory cytokine, was not modified in two *ex vivo* studies after 16 weeks of treatment with omalizumab [81, 86]. A modulation of the transcription and/or secretion of these different cytokines could thus contribute to a decrease in the recruitment and activation of the inflammatory cells involved in the late inflammatory stage of asthma and reduce long-term remodelling of the airways [87].

In addition to the above, omalizumab has a preventive effect on viral-induced exacerbations in children with allergic asthma, since blocking IgE decreases susceptibility to rhinovirus infections and illness [88]. Dendritic cells play a crucial role in innate immune defence against infections, particularly viral infections [89]. During the respiratory allergic response, dendritic cells ensure the presentation of antigens to T lymphocytes and are also capable of polarizing naïve T lymphocytes in Th2 lymphocytes [90]. Dendritic cells express the FcεRI receptor on their surface, such as basophils and mast cells [91]. The binding of IgE to dendritic cells inhibits their antiviral capacities [92, 93]. A decrease in the expression of FcεRI on dendritic cells induced by omalizumab may enhance antiviral immune responses and participate in the prevention of a significant number of asthma exacerbations as demonstrated [88].

2.2. Treatment Options in Severe Nonallergic Asthma. Patients with nonallergic asthma are usually more severe and require higher doses of ICS to control symptoms, which may reflect the fact that there may be a degree of corticosteroid resistance as a result of superantigen exposure and activation of MAP kinase pathways [15, 24]. Although patients with severe asthma represent "only" 10% of asthmatic patients, they are the most challenging and with most impairment of quality of life and absenteeism [1, 8, 19].

Severe asthma patients with a non-Th2 phenotype with sputum neutrophilia might benefit from macrolide therapy [16]. A very recent study showed that azithromycin reduced asthma exacerbations in both severe eosinophilic and noneosinophilic asthma, suggesting an immunomodulatory effect of macrolides [94]. This immunomodulatory effect may be a possible mechanism of action of omalizumab in both eosinophilic and noneosinophilic asthma. On the other hand, patients with nonallergic but with clear high-Th2 features might be considered good candidates for biotherapies against IL-5, such as mepolizumab or reslizumab [69–71].

In nonallergic asthma, there is frequent elevation of total IgE, including at the bronchial tissue level [95] and it is now established that dendritic cells participate in its pathophysiology [96, 97]. As in allergic asthma, omalizumab reduces the expression of FcεRI on the dendritic cells of nonallergic asthma patients [12]. It is likely that other cells expressing FcεRI involved in the pathophysiology of certain nonallergic asthma phenotypes are targeted by omalizumab [98]. Evidence and especially good quality evidence is emerging regarding the efficacy and safety of off-label uses of omalizumab in severe nonallergic asthma [12, 24, 53, 68, 99–106].

The field of action of omalizumab is therefore not limited to a simple anti-IgE activity. The molecule can inflect airway remodelling on one hand and induce clinical efficacy in non-allergic pathologies, but the mechanisms of action at the cellular and cytokine level, anti-Th2 and anti-inflammation, still need to be clarified. In-depth knowledge of the mechanisms

of action of omalizumab would make it possible to identify predictive biomarkers of efficacy, which are valuable in the phenotyping and therapeutic management of patients with severe asthma.

3. Conclusions

Although no good quality evidence is currently available to determine which patients with severe nonatopic asthma should be selected for omalizumab treatment, some issues should always be kept in mind: (a) the diagnosis of nonatopic asthma is not easy and should be carefully confirmed; (b) the definition of severe asthma is heterogeneous and should always be carefully assessed; (c) biomarkers may be helpful to select subsets of patients which might benefit from omalizumab treatment; (d) poor adherence and comorbidities, mainly obesity, interact negatively with asthma and should always be addressed with specific pharmacological and nonpharmacological measures. Based on literature and clinical experience of the authors, there is a clear benefit for allergic asthma patients to be treated with omalizumab. Moreover, those patients with severe nonatopic asthma (including those with high FeNO as a marker of IL-13 inflammation, high eosinophils, and periostin), uncontrolled besides optimal nonpharmacological and pharmacological treatment, **may benefit from omalizumab** therapy. However, when to suspend omalizumab according to response to therapy in each patient and pharmacoeconomical analysis are questions that remain to be answered.

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Conflicts of Interest

Cláudia Chaves Loureiro reports collaborating and receiving fees from Astra-Zeneca, GlaxoSmithKline, Novartis, Menarini, Mundipharma, Pfizer, and TEVA, through either participation in advisory board or consultancy meetings or congress symposia. Luís Amaral declares collaborating and receiving fees from Novartis. José Alberto Ferreira declares collaborating and receiving fees from Astra-Zeneca, GlaxoSmithKline, Laboratórios Vitória, Novartis, and Sanofi, through either participation in advisory board or consultancy meetings or congress symposia. R. Lima declares collaborating and receiving fees from Astra-Zeneca, Novartis, Mundipharma, GlaxoSmithKline, Menarini, and TEVA, through either participation in advisory board or consultancy meetings or congress symposia. Cecília Pardal declares collaborating and receiving fees from Astra-Zeneca, Novartis, TEVA, Menarini, Mundipharma, Pfizer, Tecnifar, and Bial. Ivone Fernandes declares collaborating and receiving fees from TEVA, Novartis, Menarini, Astra-Zeneca, Novartis, and Tecnifar. Luisa Semedo declares collaborating and receiving fees from TEVA, Novartis, Menarini, and Pfizer. Ana Maria Arrobas declares collaborating and receiving fees from Astra-Zeneca, Novartis, TEVA, Mundipharma, and Roche, through either participation in advisory board or consultancy meetings.

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Review Article

Precision Medicine in Targeted Therapies for Severe Asthma: Is There Any Place for "Omics" Technology?

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According to the current guidelines, severe asthma still represents a controversial topic in terms of definition and management. The introduction of novel biological therapies as a treatment option for severe asthmatic patients paved the way to a personalized approach, which aims at matching the appropriate therapy with the different asthma phenotypes. Traditional asthma phenotypes have been decomposing by an increasing number of asthma subclasses based on functional and physiopathological mechanisms. This is possible thanks to the development and application of different omics technologies. The new asthma classification patterns, particularly concerning severe asthma, include an increasing number of endotypes that have been identified using new omics technologies. The identification of endotypes provides new opportunities for the management of asthma symptoms, but this implies that biological therapies which target inflammatory mediators in the frame of specific patterns of inflammation should be developed. However, the pathway leading to a precision approach in asthma treatment is still at its beginning. The aim of this review is providing a synthetic overview of the current asthma management, with a particular focus on severe asthma, in the light of phenotype and endotype approach, and summarizing the current knowledge about "omics" science and their therapeutic relevance in the field of bronchial asthma.

1. Introduction

Severe asthma management still represents a matter of debate, due to asthma heterogeneity and complexity. Today, asthma is classified and assessed according to both phenotypes and endotypes approaches. The last reflects the complex interaction of inflammatory molecules and multiple pathways and systems that are involved in the pathogenesis of asthma. The use of omics technologies represents an effective way for better exploring and defining asthma endotypes. More in general, the omics approach and the application of systems biology methods provide unbiased tools allowing for better understanding of asthma pathophysiology and for developing "precision medicine" approaches. In contrast with the more general but still used "one size fits all" approach, precision medicine consider a specifically targeted therapy that includes specific biological profiles together with patient's exposure and lifestyle. The omics technologies are contributing to the identification of new biomarkers that compose these biological profiles and consequently to the development of targeted biological therapies. For a decade, omalizumab has been the only available therapy for severe allergic asthma. Recently, new promising drugs such as mepolizumab and reslizumab have been introduced as a targeted treatment option for Eosinophilic asthma.

The aim of this review is to offer an overview regarding the management of asthma, from phenotype to inflammatory endotypes. We will focus on pathophysiology mechanisms of severe asthma and on new treatment options based on different endotypes. We will finally discuss the current knowledge about "omics" science and its relevance in exploring new biological endotypes, which will represent the basis for the

development of new promising asthma therapies. The transition from new biomarkers discovery and understanding and the development of new successful therapies is still very difficult. Our review will help the clinician to understand how it will be possible to improve the management of severe asthma thanks to the most advanced research tools and what to do to optimize what is already available.

1.1. Data Collection Strategy. For this review, a highly sensitive search strategy has been developed, and validated keywords filters have been applied to retrieve articles pertaining to severe asthma definition and management.

In particular a selective search on PubMed and Medline was carried out, and research papers, international guidelines, recommendations, position papers, systematic reviews, and Cochrane meta-analyses relevant to the topic have been included in the review.

We applied a search strategy for identifying the following keywords.

Keywords for part 1 are as follows: asthma phenotypes, asthma endotypes, T2-low and T2-high subtypes, targeted therapies and bronchial thermoplasty coupled with severe asthma.

Keywords for part 2 are as follows: genomics, pharmacogenomics, transcriptomics, epigenomics, proteomics and metabolomics coupled with severe asthma

To retrieve international and European large-scale projects hand searches were performed of the reference lists of all pertinent reviews and studies examined. Abstracts from relevant conferences were searched.

2. External Phenotypes and Endotypes

A phenotype is defined as the set of an organism's observable characteristics or traits, such as its morphology and development. As a basic definition, the phenotype is mainly influenced by the interactions between genomic asset and the influence of several environmental factors. At a molecular level, the phenotype is the outcome of the expression and interaction of different endotypes, which are defined by a distinct functional or pathophysiological mechanism [1].

External Phenotypes of Asthma. Asthma symptoms are traditionally defined by shortness of breath, wheeze, chest tightness, and cough. However, it is well known that there are different asthma phenotypes. Historically, bronchial asthma was classified as allergic (extrinsic) or nonallergic (intrinsic). Extrinsic atopic asthma generally develops under the age of 40, and it is triggered by inhaled allergens and is usually associated with other allergic diseases, such as rhinitis and dermatitis [2]. On the other hand, intrinsic asthma typically develops later in life (>40 years old) and is usually less recognizable. By definition, intrinsic asthma is not associated with allergic sensitization, but aspirin-intolerance often triggers disease exacerbations. Nowadays, asthma phenotyping also includes several clinical information such as age, concomitant comorbidities (obesity, allergic rhinitis, and sinusitis), exacerbations factors (exercise, allergens, and infections), and response to the treatment.

Endotypes. An endotype is specifically defined by the pathophysiologic mechanisms underlying the phenotype(s). The management of severe asthma is benefitting from the characterization of an increasing number of different endotypes, which represent the targets of specific therapies [3].

3. T2 Subtypes

Asthma phenotyping based on inflammatory cell count (Eosinophilic, Neutrophilic, and Paucigranulocytic) in tissue and blood is gaining an increasing interest. Nowadays, two main subtypes of type 2 inflammation have been defined: T2-high (T helper type 2 cell high) and T2-low (T helper type 2 cell low) [4, 5].

The T2-high subtype is characterized by the presence of high eosinophil level in airways and includes the following: 1. early onset, allergic sensitization, responsiveness to inhaled corticosteroids (ICS); 2. late-onset, absence of allergic sensitizations, sinusitis, and lack of ICS responsiveness; 3. exercised-induced asthma.

The T2-low subtype is characterized by Neutrophilic or Paucigranulocytic airway inflammation and may consist of the following: 1. obesity-related asthma, late-onset; 2. asthma and chronic obstructive pulmonary disease overlap syndrome (ACO)/Neutrophilic, late-onset; 3. smoking-related asthma; 4. paucigranulocytic, associated with smooth muscle (Figure 1) [4].

4. T2-High

4.1. Pathogenesis and Potential Biomarkers. Most of the new biologic drugs target the Th2 cytokines pathway. These cytokines (IL-3, IL-4, IL-5, IL-9, and IL-13) are expressed in bronchial submucosa and could trigger the release of mediators that could support other inflammation patterns as well, such as thymic stromal lymphopoietin [6]. Type 2-high asthma involves different important inflammatory cells including type 2 innate lymphoid cells, Th2 cells, natural killer T cells, and mast cells. Cytokines contribute to the activation and recruitment of immunoglobulin (Ig) E antibody-producing B-cells, which sustain the allergic airway inflammation. Recently, McKenzie and colleagues described group 2 lymphoid cells producing these cytokines, defining another pathway which contributes to the T2 high profiling (Figure 1) [7]. At present, several biomarkers can identify inflammatory characteristics of T2-high endotypes (serum IgE, serum periostin, blood eosinophil, and exhaled nitric oxide eNO) both for adult and in children asthma (Table 1) [8]. However, the most validated method to assess airway inflammation is currently the sputum cytometry. At present, four inflammatory patterns can be defined based on the granulocytes detected in the sputum: 1. Eosinophilic, 2. Neutrophilic, 3. Mixed-granulocytic (both neutrophils and eosinophils are elevated), and 4. Paucigranulocytic (neither neutrophils nor eosinophils are elevated).

According to several studies, Eosinophilic asthma is defined by the presence of elevated sputum eosinophil count (>3% with or without degranulation) and/or blood

Table 1: **Overview on asthma biomarkers.**

BIOMARKER	ENDOTYPE	ACTIVATED CYTOKINES	ROLE IN INFLAMMATION PATHWAY	BIOLOGICAL AGENTS
IgE (serum)	**T2 high**: Allergic	IL-4, IL-13 through activated Th2 cells	Binds FcεRI expressed on the surface of mast cells, eosinophils, basophils and B lymphocytes Leads to subsequent degranulation and release of mediators	Omalizumab
Eosinophils (serum and sputum)	**T2 high**: Eosinophilic	IL-5	Involved in production of reactive oxygen species, desquamation and lysis of airway epithelial cells Promote airway remodelling	Mepolizumab, Reslizumab, Benralizumab
Surrogate periostin (serum, sputum)	**T2 High:** Eosinophilic-Allergic	IL-4, IL-13	Induce an amplification and persistence of chronic inflammation of allergic diseases Involved in the process of subepithelial fibrosis in asthma patient and in airway remodelling	Lebrikizumab, Tralokizumab, Omalizumab
Exhaled nitric oxide (FeNO)	**T2 high**: Allergic	IL-4, IL-13	Useful surrogate of airways inflammation Due to increased nitric oxide production by activated bronchial epithelial cells	No biological agents, but guideline recommended therapies
Dipeptidyl peptidase 4 (DPP-4 serum)	**T2 High:** Eosinophilic	IL-13	Induces the proliferation of airway smooth muscle cells, lung fibroblasts and fibronectin production	Tralokinumab
Galectin-3 (bronchial tissue)	**T2 high**: Allergic	No target identified	Involved in eosinophil recruitment, airway remodelling and development of Th2 phenotype Early predictive biomarker of modulation of airway remodelling in severe asthma patients treated with omalizumab.	Omalizumab
Neutrophils (sputum)	**T2 low**: Neutrophilic/Paucigranulocytic	IL-8	Induce the release of O2, matrix metalloproteinase-9 (MMP-9), leukotrienes-4 (LTB-4), and platelet-activating factor (PAF)	No biological agents still available

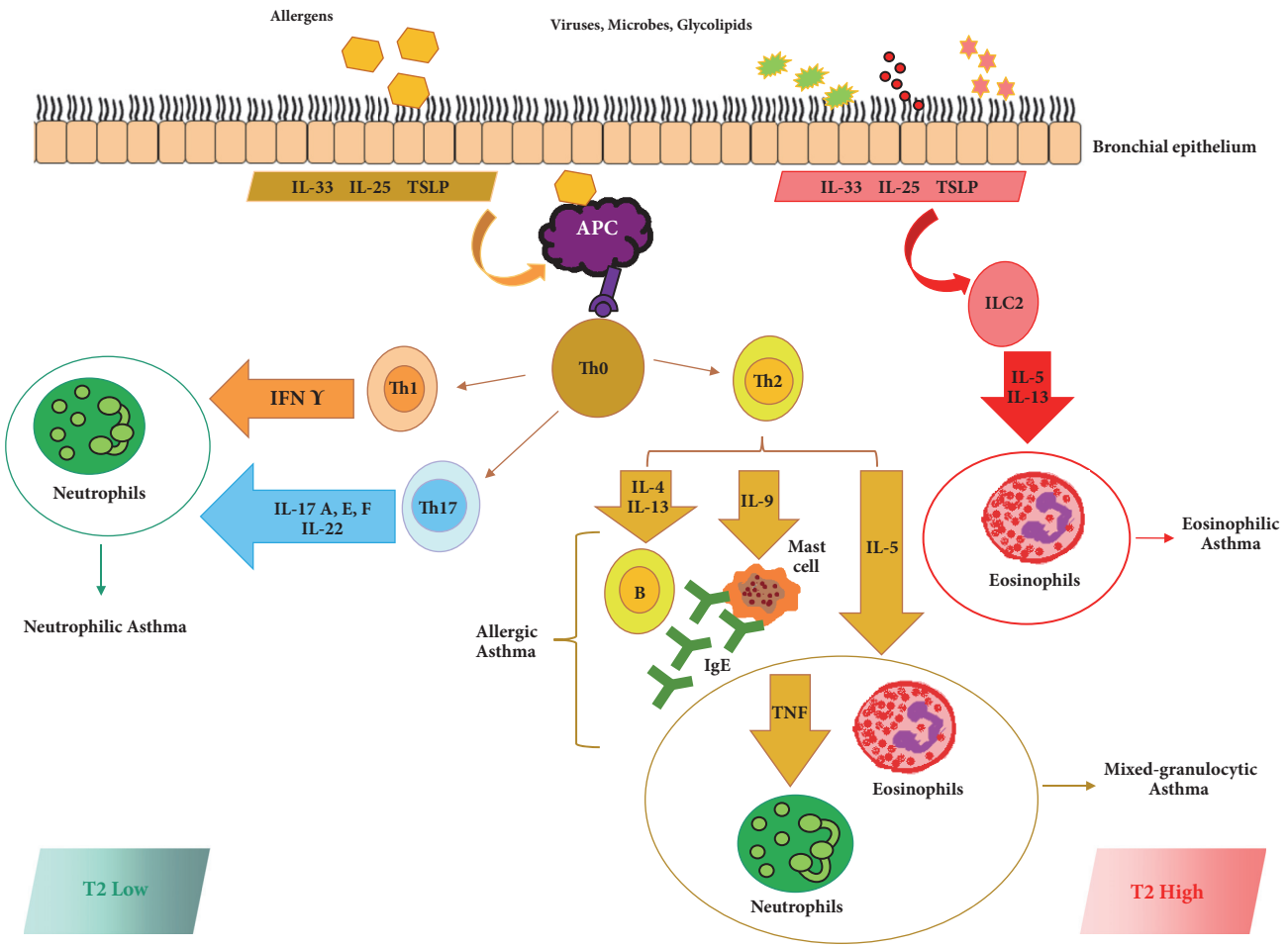

Figure 1: T2-high and T2-low asthma pathway. The T2-high subtype is characterized by the presences of high level of eosinophils in airways, and the T2-low subtype is characterized by Neutrophilic or Paucigranulocytic airway inflammation. APC antigen presenting cell, ILC2 type 2 innate lymphoid cells, TSLP thymic stromal lymphopoietin, and TNF (tumor necrosis factor).

eosinophil count (≥400 cells/μL) detected at least in two consecutive controls and by symptoms and exacerbations control obtained with treatment aimed at suppressing eosinophils [9]. However, for the other three patterns, there is no indication of minimum thresholds.

4.2. Targeted Therapy. In recent years, the therapeutic options that target the T2-high subtype have been significantly increasing. At present, the best biomarker predicting a good response to anti-IL-5, anti-IgE, anti-IL-4/IL-13, corticosteroids, and receptor for prostaglandin D_2 (CRTH2) is the blood eosinophils count, while periostin and dipeptidyl peptidase-4 (DDP-4) can predict response to anti-IL-13 [5].

IgE Blockers. Omalizumab, the first biological approved for asthma, is a humanized monoclonal antibody (mAb), approved in 2003 by US Food and Drug Administration (FDA). It depletes IgE antibodies and blocks their action on effector cells, by reducing the density of high-affinity IgE receptors [10]. Omalizumab is effective in patients aged 6–75 years with allergic asthma and sensitized to perennial allergens and present levels of IgE serum ≥30UI/mL≤1500 UI/m. Omalizumab showed efficacy and safety in randomized clinical trials (RCTs) and real-life setting, in terms of reduction of exacerbation rates and steroid-sparing effect [11]. Furthermore, it indirectly decreases airway eosinophilia and for this reason it is more effective in patients with higher levels of exhaled oxide nitric, blood eosinophils, or blood periostin [11, 12]. These combined biomarkers also showed a predictive value for clinical response, and discontinuation of anti-IgE treatment in patients with these features demonstrated a more rapid loss of asthma control [13]. Severa studies confirm that a long-term treatment with omalizumab allows an improvement of symptom control and a sustained reduction of exacerbation risk in adult patients. [13, 14]. On the opposite, there is still a lack of biomarkers that can guide the clinician in continuing or suspending treatment with patient growth in pediatric populations. However, Baena-Cagnani and coworkers showed that omalizumab may have a disease-modifying effect in children with moderate/severe uncontrolled asthma. During the first 3 years of follow-up, after the treatment with this drug, they were completely free of asthma symptoms [15].

Quilizumab, a humanized IgG1 mAb, targets the M1-prime segment of membrane-expressed IgE causing the depletion of IgE-switched and memory B-cells. Unfortunately, clinical studies did not confirm a significant clinical efficacy in patients with uncontrolled refractory allergic asthma [16]. Ligelizumab (QGE031), an IgG1k anti-IgE mAb, has a major suppressor effect on free IgE compared with the gold standard omalizumab, with a better pharmacodynamic effect in allergic subjects, even in the case of higher IgE levels. These improvements could allow a successful treatment in patients who show inadequate response or unresponsiveness to omalizumab [17].

Anti-IL-5. Mepolizumab and reslizumab are two mAbs that bind IL-5 with high specificity and affinity and have been recently approved for the treatment of severe uncontrolled Eosinophilic asthma. Mepolizumab is a N-glycosylated IgG1/k humanized mAb, approved as an add-on subcutaneous therapy in patients aged at least 18 years with severe Eosinophilic asthma and blood eosinophil levels of 300 cells/mcL or greater and 150 cells/mcL during the previous 12 months [18]. Mepolizumab showed efficacy in the reduction of exacerbations, it exerts an oral glucocorticoid-sparing effect, and it determines the improvement of quality of life. However, data on the increase of Forced Expiratory Volume in 1 Second (FEV1) were contradictory except in the MUSCA study [19]. Reslizumab, an IgG4/k mAb, has been approved as an add-on intravenous monthly treatment in patients aged at least 18 years with severe Eosinophilic asthma, with baseline blood eosinophilia ≥400 cells/μL [20]. It improves asthma control, FEV_1, and quality of life (QoL) [21, 22]. However, there are still concerns about the route of administration and the real positioning of this drug in the general context.

Benralizumab, an anti-eosinophil mAb approved in 2017 by FDA, is an IgG1/k antagonist of the α chain of human IL-5 receptor [23, 24]. This drug is the only one that can induce apoptosis by means of cellular toxicity mechanisms (antibody-dependent cell-mediated cytotoxicity or ADCC) in its target cells, reducing the level of eosinophils in tissues by 90–100. Furthermore, its clinical effect is independent of the IL-5 circulating levels, which usually tends to increase during asthma exacerbations [25]. Data from RCTs confirmed the efficacy of benralizumab in reducing annual exacerbations rates and improving FEV1. Moreover, benralizumab showed a significant systemic steroid-sparing effect [26, 27].

Anti-IL-4, AntiIL-13. Dupilumab, a fully humanized mAb anti-IL-4 receptor currently investigated in phase 3 studies, inhibits the biologic effect of both IL-4 and IL-13 by preventing their interaction with IL-4 receptor α subunit. Several studies have demonstrated its efficacy in the reduction of asthma exacerbations and improvement of symptoms, QoL, and respiratory function [28, 29] irrespective of their baseline blood Eosinophilic count. There are still doubts about its safety profile, in particular regarding the evident rise of blood eosinophil levels which happens predominantly in patients with asthma and elevated baseline serum eosinophilia [30].

Antibodies targeting free circulating IL-4 (pascolizumab, altrakincept) [31, 32] or IL-13 (anrukinzumab, IMA-026, GSK679586) have been studied and appear safe and tolerable, but they have been discontinued due to failure in reaching primary outcomes [33–35].

Lebrikizumab and tralokinumab, two IgG4 anti-IL-13 mAb binding free-IL-13, are still under development but, so far, did not show any clinical improvement in asthma exacerbation rate and only a modest clinical effect has been demonstrated [36, 37].

Novel Therapies. Several other drugs are currently under development, including antithymic stromal lymphopoietin (TSLP) such as AMG157-tezepelumab [38] that mitigates the early and the late-onset-phase responses to allergens. TSLP, IL-33, and IL-25 are key mediators of type-2 inflammation diseases (such as asthma, nasal polyposis, and Eosinophilic esophagitis); therefore they are deserving an increasing interest as potential target of new drugs. IL-25 and L-33 inhibitors have unfortunately not reached the clinical outcomes [39].

The stimulation of prostaglandin antagonist's receptor (CRTH2), present on lymphocytes, eosinophils, and basophils surface, induces chemotaxis of these inflammatory cells and the release of mediators. There is a growing interest in exploring drugs targeting this receptor (fevipiprant, setipiprant, and OC000459) and some results are emerging, such as the improvement in FEV_1 demonstrated by Pettipher et al. when a specific CRTH2 antagonist was used for patients with eosinophils count > 250 cells/μL [40, 41].

New interest is emerging around interferon, as it is well known that respiratory viruses, especially rhinovirus, are implicated, not only in asthma exacerbations but also in the pathogenesis of asthma and Th2 inflammation. A phase 2 RCT with IFN-β treatment has been shown to be effective in enhancing innate immunity both systemically and in the lung (it has been demonstrated by serum concentration of CXCL10 as well as through the improvement of morning peak-expiratory flow (PEF)) in severe asthmatic patients [42]. The nebulized IFN-β treatment seems to act on the viral-response pathway and, administrated at the early onset of cold symptoms, prevents worsening of asthma symptoms.

T2-high blockers are responsible for clinical benefit in many patients with T2-high asthma but this may cause recurrence of the symptoms [43], so that a true immunomodulation has not yet been demonstrated. However, there is still the need of further studies that involve larger group of patients to detect and evaluate new endotypes, particularly for patients that are unresponsive to the treatments so far available.

The modern medicine is increasingly moving towards precision therapy, because it is unlikely that one therapeutic approach will be able to offer clinical benefit to all T2-high asthmatic patients. Clinical trials will benefit from a careful assessment of the targeted pathway and this should be reached by means of molecular phenotyping approach.

5. T2-Low

5.1. Inflammatory Mechanisms. Neutrophilic asthma is characterized by elevated neutrophils (≥64%), but not eosinophils

(<3%), by increased total cell count (≥9.7 million cells/g) detected at least two times, and by unresponsiveness to treatments suppressing eosinophils.

Mixed-granulocytic asthma is identified when there is evidence of both neutrophils and eosinophils on at least two detections, independently or concurrently.

Paucigranulocytic asthma has low eosinophils (<3%) and low neutrophils (<64%). In that case treatments aimed at suppressing both inflammatory patterns are ineffective in controlling symptoms [3]. On the basis of inflammatory mechanisms, two different patterns can be identified: Th1 and Th17. Th1 cells release IFNγ, which is involved in intracellular infections and autoimmunity. Th17 cells are CD4+ T lymphocytes expressing IL-17A, IL-17E, IL-17F, and IL-22, which can activate neutrophils through the production of IL-8 (Figure 1) [44]. Airway damage associated with Neutrophilic inflammation leads to mucus gland hyperplasia and hypersecretion, airway hyperreactivity, remodelling, and corticosteroid insensitivity [45].

Regarding airway remodelling, it can be considered as a result of an impaired mucosal repair process, characterized by increased airway smooth muscle mass, subepithelial fibrosis, and increased number of mucous glands and goblet cells caused by Th2 cytokines as well as by growth factors and cytokines produced by epithelial cells and macrophages. These structural modifications alter airway mechanism and contribute to airway hyperresponsiveness [44].

5.2. Pathogenesis and Treatment Options. T2-low field represents a new evolving research area, and to date there are no effective therapies. This endotype is characterized by non-Eosinophilic airway inflammation. It occurs in nearly 50% of patients with asthma [46, 47]. T2-low can be subdivided into Neutrophilic, characterized by mediators implicated in the pathogenesis of Neutrophilic inflammation, such as IL-8, IL-23, and IL-17, and Paucigranulocytic inflammation. These patients show not optimal response to corticosteroids, but they have demonstrated good responsiveness to a group of antibiotics, macrolides (azithromycin and clarithromycin). The Neutrophilic inflammation in asthma may be due to corticosteroids treatment inducing impaired apoptosis of neutrophils and Th17-mediated Neutrophilic inflammation, pulmonary infections, smoking habit or occupational exposition, and altered airway microbiome [48].

Therapies for non-Eosinophilic inflammation may include macrolides, statins, and theophylline but data are still controversial [49, 50].

Other novel small molecules targeting Neutrophilic inflammation were investigated, such as C-X-C-chemokine receptor (CXCR2) antagonists, CXCL8 (IL-8), and peroxisome proliferator-activated receptor-γ (PPARγ) [51]. Published results are contradictory [52, 53].

As for the monoclonal antibodies brodalumab and secukinumab (both anti-IL-17A), no improving in asthma symptoms has been shown [54] and daclizumab (anti-CD-25) is effective in improving symptoms and function, but it is unclear to which patients it should be addressed [55]. Studies that investigated TNFα blockers (etanercept, golimumab) did not demonstrate a significant clinical effect in treated patients [56, 57]. Some other chemokines could be targeted, including IL-1β or IL-6 [58] FLAP (5-lipoxygenase-activating protein) inhibitors such as GSK-2190915, which prevents the formation of LTB4 [59], involved in response to allergen. No active clinical trial is currently in development.

Phosphodiesterase (PDE) and Protein Kinase Inhibitors. PDE4 inhibitors and dual PDE3 and PDE4 inhibitors exert immune-modulatory effect potentially effective on asthma inflammation. RPL554 is a molecule registered in a clinical trial for the treatment of asthma and COPD [60]. Proteins kinases are involved in the cellular pathway of proinflammatory cytokines. Different molecules are under development [61], including PIK3 kinase inhibitors. PIK3 inhibition partially shares the mechanisms of action of low dose of theophylline [62] and some studies have demonstrated a potential effect in restoring corticosteroid sensitivity [63].

There are no active phase 3 clinical trials for the target molecules of the T2-low type. It seems that the knowledge on the pathophysiology of this endotype is still poor and no treatments are currently available. Understanding the biology and the pathophysiology of the disease will require a closer collaboration between clinical specialists and biologists, in a multidisciplinary effort.

6. Bronchial Thermoplasty

Bronchial thermoplasty (BT) is a nonpharmacological endoscopic procedure based on controlled heat release. The potential effect is an appreciable change in airway wall structure, by reducing the amount of smooth muscle with a device called Alair™ Catheter (Boston Scientific, Natick, MA, USA). BT is delivered in 3 short sessions, and no incisions or full anaesthesia is necessary. Each session is routinely performed under deep sedation administered by an anaesthesiologist and typically it takes 30–40 minutes to be completed. The procedure consists in the treatment of right lower lobe, left lower lobe, and right and left upper lobe in three different sessions. Sessions are performed every three or four weeks.

BT was approved by FDA in 2010 and according to last ERS/ATS guidelines it is recommended in adults with severe refractory asthma after approval by an Institutional Review Board [64]. The mechanism of action remains unclear. The literature reports reduction of ASM as a BT target [65]. Recently our team demonstrated a reduction of nerve fibers in epithelium and ASM. This result could explain the clinical improvement of patient that underwent BT [66].

Today literature addressing BT treatment of the T2-low endotype characterized by Neutrophilic or Paucigranulocytic airway inflammation is poor [3]. In our experience, patients were included into BT pathway first in the context of a clinical trial and subsequently as a clinical practice procedure.

Patients were also enrolled for BT treatment when not responsive to mAbs. Moreover, BT may be considered as a preferential treatment for patients who could not be addressed to other therapies, or who decided to perform a once-in-a-lifetime therapy [67]. On the basis of our experiences and clinical data, BT shows long-term effectiveness [68]

and, therefore, it should be considered not only an experimental procedure but rather an important treatment option for adult patients with severe asthma [69].

7. Biomarker Discovery: Through the Detection of Novel Endotypes

Omics are a neologism that defines a new "global" molecular biology point of view that through a single analysis can characterize large-scale members of biochemical pathways and molecular functional activities. From DNA microarray to Next Generation Sequencing (NGS), the omics sciences are increasing, and they involve new techniques and approaches in order to better understand the disease and therefore enabling more effective drugs and therapies [70].

Omics technology is providing new biomarkers that may be used as novel targets for diagnostic tests and pharmacologic treatments. This will happen through the increasing of the knowledge of biological mechanisms and the microenvironment of asthma inflammation. This path is moving forward to the development of "precision medicine" approaches [70, 71].

Omics are contributing to help precision medicine to identify the right therapy to the proper clinical phenotype. The added value of omics technologies is particularly evident in severe asthma studies aimed at identifying novel endotypes.

Suffix "ome" derives from "chromosome" and today includes genomics, transcriptomics, proteomics, metabolomics, and epigenomics (Figure 2).

Genomics is a branch of genetics that studies the sequencing and analysis of an organism's genome.

Transcriptomics is the study of complete set of RNA transcripts that are produced by the genome.

Proteomics refers to the systematic identification and quantification of the complete complement of proteins (the proteome) of a biological system (cell, tissue, organ, biological fluid, or organism).

Metabolomics concerns the scientific study and analysis of the metabolites produced by a cell, a tissue, or an organism.

Epigenomics is the study of all of the epigenetic changes in a cell [72].

8. Large-Scale Projects and the Development of Databases

To date several international projects that involve hundreds of adult and children asthmatic patients have been developing in several countries, in order to better understand severe asthma, determine differences among asthma patients, and gain new finding to create new therapeutics options.

One example of these projects is the Severe Asthma Research Program (SARP), supported by the NIH (National Heart, Lung and Blood Institute) in the United States. This program is a network enrolling over 700 patients both adults and children coming from several states [73].

Another example is the "Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes" (U-BIOPRED). This is a European project involving 16 centers in 11 European countries. The goal of this large-scale dataset is to increase the numbers and types of asthma biomarkers by integrating clinical data with inflammatory biomarkers derived from omics. U-BIOPRED provided an unbiased algorithm that trough system biology technology matches an exponential quantity of data that will enable phenotyping severe asthma and will pave the way to new tailored therapeutic approach [74, 75].

Within the European Union, national databases have been starting to develop. For example, Italian researchers have recently created the Severe Asthma Network in Italy (SANI). It is a multicenter register, which involves referral centers for the treatment of severe asthma. Up to now 549 adults and children patients with different types of asthma have been included [76, 77]. The aim of this registry is to identify and characterize patients eligible for biological treatments (biomarkers evaluation and causal-endotype identification), to evaluate cost/benefit optimization in the field of new and traditional treatments, and to investigate treatment adherence and its determinants. SANI Network provides the opportunity to create international collaborations with networks of clinical researchers, in the respiratory field, in the framework of the SHARP program (Severe Heterogenous Asthma Research Collaboration, Patient Centers) or ISAR (International Severe Asthma Registry) [78].

Many other countries have activated severe asthma registries, such as United Kingdom, Belgium, Germany, and Australia. These initiatives are providing a huge amount of information for large-scale experiments, with the hope that this data will be freely accessible [79–82].

9. Genomics of Asthma

Different genes have been associated with asthma severity. Genome-wide association studies (GWASs) contributed to identifying several asthma risk loci. To date, several studies have been performed trying to link the disease with portions of the genome through the use of a high number of Single Nucleotide Polymorphisms (SNPs) [83]. A major study of the GABRIEL consortium in Europe involved 10,365 patients and 16,110 healthy controls who were where genotyped of 582,892 SNPs. One of the major findings was the identification of the *ORMDL3* (ORM1-like protein 3) and the *GSDMB* (Gasdermin-B) genes within 17q21 locus and *CDK12* (Cyclin-dependent kinase 12) as candidate genes for childhood-onset severe asthma [84]. The same 17q21 locus was also associated with asthma exacerbations, and treatment response, and *ORMDL3* was proposed as candidate gene [85]. *ORMDL3* negatively regulates the expression of IL-2. Considering the role of IL-2 in the differentiation of TH2 cell subsets, this effect on IL-2 production could represent a genetic risk for asthma and autoimmunity [86]. As a major finding of these projects, asthma onset has been associated with a number of genes coding for HLA, IL-13, IL-33, thymic stromal lymphopoietin [TSLP], IL-1 receptor-like 1 [IL-1RL1], ST2, and the receptor for IL-33. Furthermore RAR-related orphan receptor A [RORA], SMAD family member 3 [SMAD3], and GATA3 were identified [87]. *CDHR3* gene has also

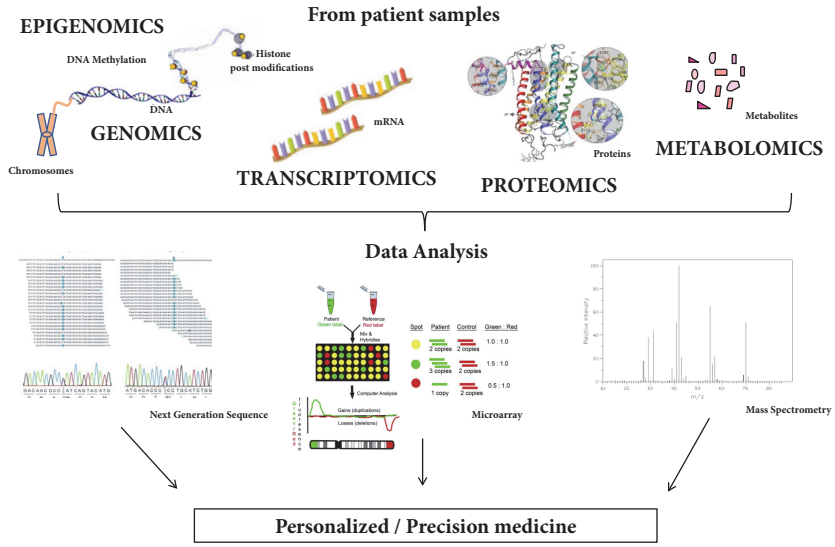

Figure 2: From omics technology to personalized medicine. From DNA microarray to Next Generation Sequencing (NGS), system biology provides for management and data analysis. This path is moving forward to the development of "precision medicine" approaches.

been described in severe asthma in addition to *GSDMB*, *IL-33*, and IL-1RL1. Furthermore, *CDHR3* gene expression was associated with exacerbation in children asthma population from 2 to 6 years of ages [88, 89].

A study conducted on severe asthma in Italian patients found a significant association between the SNP rs848 within the *IL-13* gene and severe asthma symptoms [90]. A correlation between HLA-II genes and different asthma phenotypes has also been described. In particular, HLA-DRB1 is associated with allergic asthma, HLA-DQB1 with occupational asthma, and HLA-DPB1 with aspirin-sensitive asthma [91].

10. Pharmacogenomics of Asthma

One of the most important applications of genomic association studies outcomes for severe asthma is pharmacogenomics. This omics science studies genetic variations influencing treatment response to the most commonly used asthma therapies. For this reason, several studies have already been successfully performed. For example, BARGE (Beta-Adrenergic Response by Genotype) trial showed that patients with an ARG16 variant of ADRB2 gene had a small decline in lung function compared to Gly16gly genotype. The response to the short-acting beta-2 agonist (SABA) therapy has been correlated with a mutation in the coding sequence of the beta-2 adrenoceptor [ADRB2] gene that causes a Glycine/Arginine substitution at position 16 of the receptor protein (Gly16Arg) [92]. In line with response to SABA, pharmacogenetic works on response to long acting beta-2 agonist (LABA) have addressed the ADRB2 gene but no effect on lung function was found [93]. However, the Arg16 variant showed an impact on LABA in pediatric population [94]. Several GWASs on inhaled corticosteroids (ICS) response in asthmatic population were performed but no clinically significant results were reported [95, 96]. Although Mosteller and coworkers [95] did not find any significant genetic markers in their study, other studies have identified a novel SNP, rs10044254, associated with both decreased expression of the F-Box and Leucine Rich Repeat Protein 7 [FBXL7] gene and improved symptomatic response to ICSs in pediatric subjects [97]. These very interesting findings suggest that there might be a specific genetic mechanism regulating symptomatic response to ICSs in children not present in adults.

On the opposite, variations of the *GLCCI1* gene, encoding for glucocorticoid-induced transcript 1 protein, have been associated with a reduction of pulmonary functions. Unfortunately, these results were not confirmed by other clinical trials. The rs2872507 SNP, which influences *ORMDL3* gene expression at locus 17q21, may be a possible marker for ICS treatment response in childhood asthma [98]. The rs2872507 T variant of this gene was found in the SHARP population and is associated with an improvement in FEV1 in asthmatic patient on ICS therapy. Many clinical trials investigated the response to leukotriene modifiers, and, despite a quantity of candidate gene, only 5-lipoxygenase (ALOX-5), transporter gene (MRP1), and ATP-binding cassette (ABCC1) transporters were found to be involved in the LTRA response [98]. Despite the efforts dedicated to the investigation of genetic variations associated with asthma, so far there is no evidence of strong association with treatment heterogeneity of response and no pharmacogenetic marker seems to be able to reach clinical relevance. Further investigations are therefore needed to identify specific genetic variation influencing treatment response and to provide new distinct asthma phenotypes.

11. Transcriptomics

Bigler and collaborators recently performed a whole genome expression of blood cell in asthma. In this research, 1,693

genes were differentially expressed in severe asthmatic patients [99]. Using bronchial epithelium and induced sputum samples, several case/control studies tried to define the different asthma subtypes through differential expression of messenger RNA (mRNA). Woodruff and coworkers identified the two most popular asthma subgroups, "T2-high" and "T2-low," through the different expression of IL-5 and IL-13 transcripts in bronchial biopsies. Furthermore, epithelial expression of *POSTN* (periostin), *CLCA1* (calcium-activated chloride channel regulator 1), and *SERPINB2* gene transcripts may be predictive of a Th2 driven inflammation [100]. Shikotra et al. found an upregulation of the *CEACAM6* (carcinoembryonic antigen related cell adhesion molecule 6) transcript in bronchial biopsies of asthmatic patients; it was associated with airway epithelial cells and tissue neutrophils, showing that the *CEACAM6* expression levels could be linked to a Neutrophilic asthma phenotype [101].

In the past few years, some studies aimed at identifying a "T2 gene-based discrimination" in induced sputum samples, which is less invasive than bronchial biopsies [102]. Other studies detected seven gene transcripts [COX-2 (cyclo-oxygenase-2), ADAM-7 (disintegrin and metalloproteinase domain-containing protein 7), SLCO1A2 (solute carrier organic anion transporter family member 1A2), TMEFF2 (transmembrane protein with epidermal growth factor like and two follistatin like domains 2), TRPM-1 (transient receptor potential cation channel subfamily M member 1), and two unnamed] in bronchial brushing samples with expression levels that were moderately correlated with submucosal eosinophils [103].

Severe asthma in adults is characterized by inflammatory pathways involving mast cells, eosinophils, and group 3 innate lymphoid cells detected in induced sputum, endobronchial, and nasal brushing [104]. Baines et al. identified transcriptional inflammatory asthma phenotypes (TAPs) by studying the gene expression profile from induced sputum of adult stable asthma. Three distinct TAPs groups were identified: TAP 1 Eosinophilic, TAP2 Neutrophilic, and TAP3 Paucigranulocytic [105]. When the TAP profiles were compared with gene expression analyses of sputum, a 92% of overlap was detected.

MicroRNAs. MicroRNAs (miRNAs) are small noncoding single RNAs strands that regulate gene expression at the posttranscriptional level. MiRNAs are involved in all of the most important cells functions including the control of inflammatory processes; therefore numerous studies have been conducted to better understand the involvement of miRNAs in several diseases. The characterization of miRNAs and their role may represent an important tool for endotyping the complex asthma phenotype picture, when investigated in tissues whose collection is less invasive than common bronchial biopsies and induced sputum, such as peripheral blood.

MicroRNA expression in the peripheral blood has been investigated in a small study that compared seven mild asthmatics and four healthy subjects. This study detected an underexpression of microRNA 192 when study population underwent allergen inhalation challenge [106].

The interest in miRNA study as potential source of biomarkers is increasing. It has been shown in serum a differential expression of miR-1248 in asthmatic versus nonasthmatic patients and it has been demonstrated that miR-1248 is directly involved in the regulation of IL-5 transcript [107].

So far, severity of asthma had a minor impact on miRNA expression when it was evaluated on nasal biopsies of asthma patient [108]. However, a recent case-control study that investigated severe equine asthma identified 11 miRNAs differentially expressed. One of this miRNAs was the MiR-128 [109], which is part of a regulatory miRNA network and it has been already shown to be downregulated in bronchial epithelial cells of asthmatic patients. These results were confirmed by a significant increase of interleukin-6 (IL-6) and interleukin-8 (IL-8) that are associated with pathophysiology of asthma [110].

A preliminary miRNA study has also been performed for pediatric asthma (12 cases and 6 controls) that showed an upregulation of MiRNA-221 and miRNA-485-3p in asthmatic patient [111].

12. Epigenomics

Epigenetic mechanisms include DNA methylation, histone modifications, and noncoding RNAs, and they can control gene expression acting on DNA structure and subsequent regulation. The set of nucleic acid methylation modifications in an organism's genome is known as methylome. Several genes linked to asthma are regulated by epigenetic mechanism, such as genes involved in T-effector pathways (interferon INF-γ, interleukin-4 (IL-4), IL-13, and IL-17), T-regulatory pathways (forkheadbox P3 [FoxP3]), and airway inflammation (arginase [ARG]) [112].

A study on African American inner-city children identified 81 differentially methylated regions (DMRs) in peripheral blood mononuclear cells (PBMCs) related to allergic asthma. Several immune genes were hypomethylated including IL-13, *RUNX3,* and *TIGIT* [113].

Breton's group in 2011 studied the DNA methylation of specific genes and investigated biomarkers of airway inflammation. They found methylation levels of several CpG (regions with a high frequency of CpG site) loci located in promoter regions of ARG genes associated with FeNO. This finding could explain a possible role of DNA methylation in the regulation of nitric oxide production [114].

An epigenetic association between serum IgE levels and methylation at different loci using DNA from peripheral blood leukocytes demonstrated that genes annotated to these loci encode known eosinophil products. This finding suggests that methylation differed significantly in isolated eosinophils from subjects with and without asthma and high IgE levels [115].

13. Proteomics

The currently available literature provides several examples of detection of proteins involved in inflammatory mechanisms of asthma; they are commonly profiled using mass

spectrometry. Proteome analyses research so far has been conducted in limited sample-size studies on bronchoalveolar lavage fluid (BALF) [116, 117], bronchial biopsies [118], and sputum supernatants [119, 120]. A large-scale study stratified severity of asthma relying on granulocytes inflammatory in sputum. Patients were divided into different groups: <2% or >2% eosinophils and <40% or > 40% neutrophils. Microarray data showed different inflammatory proteins between groups [118]. The SARP group was able to identify four groups of asthma from mild-moderate to severe using the protein expression level of 18 targeted cytokines [121].

Proteomics signatures have been investigated also in biopsies of omalizumab responder (OR) versus nonomalizumab responder (NOR) phenotypes after 36 months of treatment. Baseline galectin-3 expression was found in OR patients but not in NOR. Galectin-3 detection was related to an improving of respiratory function in OR and it could be considered as a potential biomarker of long-term response to omalizumab [122].

14. Metabolomics

In order to differentiate asthma endotypes, many studies recently suggested that a measure of metabolic profiles in different samples including exhaled breath, urine, plasma, and serum may be applied [123]. Nowadays the most attractive area of metabolomics is "breathomics" [124]. It is based on the use of an electronic nose that recognizes a profile of volatile organic compounds (VOCs) in exhaled breath and is able to discriminate asthmatics from healthy controls [125, 126].

One of the studies in the field obtained a fingerprint of VOCs for asthma atopic patients by employing mass spectrometry combined with electronic nose [127].

Another small size study (25 patients) compared the eNOSE performance to sputum eosinophils and exhaled nitric oxide (FeNO). The eNOSE was able to discriminate asthmatics from healthy subjects and to predict corticosteroid response in asthmatics [126]. Furthermore, the fingerprint identified by the eNOSE correlated with the percentage of sputum eosinophils [127, 128]. Metabolomics seems to be a promising tool in identifying asthma endotypes and biomarkers; however additional studies are needed in order to enhance the knowledge and obtain a standardized approach.

15. Conclusions

From the conventional definition of asthma to the inclusion of new contemporary endotypes, science has been making huge strides in the field of asthma. To date, the endotyping dichotomy between T2-high and T2-low based on inflammatory and pathophysiology pathways improved asthma management. Currently, T2-high endotype is better understood and most of new biological therapies address T2-high asthma treatment. Among biological treatments, omalizumab has a well-documented efficacy, safety, and effectiveness. However, many studies have been conducted on other drugs targeting the T2-high pathway and this includes mepolizumab (already available on the market) and reslizumab. Both target IL-5 and their long-term efficacy is now confirmed by many studies. Benralizumab and dupilumab, addressing as well Th2-high inflammation, will be available in the near future.

On the other hand, the pathogenesis and pathophysiology of T2-low endotypes remain so far unclear and treatment options specifically addressing T2-low pattern are still lacking, except for BT.

Although the knowledge concerning asthma mechanisms is increasing, biomarkers research needs to be improved, in order to identify molecules univocally selective for the appropriate therapies and predicting response to treatment. Achieving that goal is much more needed in the frame of mAbs sustainability.

A great support could come from the omics technologies. This is a new way to approach science and data analysis. Omics technologies are facilitating rapid advance in understanding the molecular details of asthma pathogenesis and pathophysiology. It implies a commercial counterpart both in pharmaceutical and in biotechnology research, aimed at offering system biology solutions to drug developers and diagnostics companies.

Although the interest in omics technologies is increasing, several limitations still restrict their wide clinical use. Indeed, none of the above-mentioned omics signatures have been translated into clinical practice. Large-scale studies and specific RCTs are necessary in order to find a real clinical utility and application of omics science as a biomarker and prognostic factor. For example, a study conducted in a cohort of 194 asthmatic patients identified 6 clinical and pathobiological clusters based on blood and induced sputum measures [129].

From the omics, circulating miRNA deserves a specific interest. Circulating miRNAs might be a noninvasive biomarker useful to diagnose and characterize asthma. Hyperlink's team studied the expression of miRNA in the blood of asthmatic patients compared with nonasthmatic patients. Their results showed a subset of circulating miRNA (miR-125b, miR-16, miR-299-5p, miR-126, miR-206, and miR-133b) expressed in patients with allergic rhinitis and asthma [130].

The power of the new miRNA's technology consists in easy sampling, exposing the patient to the lowest possible risk and the cheap and reproducible method of quantifying miRNA blood levels.

The recent Italian register, as well as the European (U-BIOPRED) and American (SARP) ones, can represent an excellent source for data and future studies. T2-low endotypes management represents the most urgent unmet need to be addressed through the use of these new technologies, which however provide a formidable support for better understanding and treating any asthma type.

We need to consider that real-life applicability of omics technologies is still far from the levels that could be expected. In the last two decades we have witnessed an exponential increase of biological therapies in oncologic fields and regarding therapies for treatment of rheumatic diseases, solid, and blood cancer. On the opposite, in asthma area from 2006 to 2017 omalizumab has been the only available mAb. Recently, both clinical and preclinical researches have literally exploded.

Indeed only in recent years have severe asthma therapeutics options expanded their potential thanks to development of new drugs.

Despite an increasing interest in omics technology, we need to take into account the fact that none of the omics signatures mentioned above has been translated into clinical practice and that it is one of the major limits. For this reason, development of large-scale studies is urgently needed. Particularly, specific Randomized Controlled Trials (RCTs) would be necessary to definitively confirm the clinical relevance of omics and reinforcing omics role in searching for new biomarkers and prognostic factors. The need for correctly selecting the right mAb for the right patient is one of the key points in severe asthma management.

The challenge in the "omics era" is to translate from bench to bedside this huge amount of data coming from the above-mentioned dataset. Translation in clinical practice through RCT is needed to emphasize omics' role in precision medicine and to predict response to treatments.

Finally, the study of the interaction between the different biomarkers will be extremely important for better understanding asthma progress and evaluating the possible negative impact of some therapies. For these purposes, the application of mathematical models that gather the interaction of the different biomarkers will provide a great help, as well as the application of machine learning approaches that will help to decide the most successful therapies. These promising fields seem to be still far from application in the asthma field, but we are confident they will be widely investigated in the upcoming years, similarly to other medical fields.

Conflicts of Interest

Francesco Menzella participated in contracted research and clinical trials for Novartis and Sanofi and has received lecture fees and advisory board fees from AstraZeneca, Boehringer-Ingelheim, Chiesi, GlaxoSmithKline, Mundipharma, and Novartis. Nicola Facciolongo served as a consultant for Boston Scientific and has received lecture fees from Astra-Zeneca and Chiesi. The other authors report no conflicts of interest in this work.

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Review Article

Benralizumab: From the Basic Mechanism of Action to the Potential Use in the Biological Therapy of Severe Eosinophilic Asthma

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Asthma is a very frequent chronic airway disease that includes many different clinical phenotypes and inflammatory patterns. In particular, eosinophilic bronchial inflammation is often associated with allergic as well as nonallergic asthma. The most important cytokine involved in the induction, maintenance, and amplification of airway eosinophilia in asthma is interleukin-5 (IL-5), released by both T helper 2 (Th2) lymphocytes and group 2 innate lymphoid cells (ILC2). Hence, IL-5 and its receptor are suitable targets for selective biologic drugs which can play a key role in add-on treatment of severe eosinophilic asthma refractory to corticosteroids. Within such a context, the anti-IL-5 monoclonal antibodies mepolizumab and reslizumab have been developed and approved for biological therapy of uncontrolled eosinophilic asthma. In this regard, on the basis of several successful randomized controlled trials, the anti-IL-5 receptor benralizumab has also recently obtained the approval from US Food and Drug Administration (FDA).

1. Introduction

Asthma is a chronic obstructive airway disorder characterized by inflammatory and structural changes which cause a usually reversible airflow limitation responsible for recurrent episodes of dyspnea, wheezing, and chest tightness [1–4]. This very common disease affects more than 300 million people worldwide and originates from complex interactions between genetic and environmental factors [2]. The resulting phenotypes/endotypes include different patterns of airway inflammation, among which the eosinophilic subtype is quite frequent. Indeed, although the exact prevalence of eosinophilic asthma is not known, among patients with severe asthma who represent about 5–10% of the entire asthmatic population, eosinophilia in either sputum (≥2%) or blood (≥300 cells/μl) can be detected within a 32–40% range [5, 6]. In many asthmatic patients, airway eosinophilia develops as a consequence of the biological activity of both Th2 and ILC2 cells, which are crucially implicated in the pathogenesis of type-2 inflammation underlying eosinophilic allergic and nonallergic asthma. Differently from neutrophilic asthma mainly sustained by non-type-2 mechanisms driven by Th1 and especially Th17 cells, type-2 eosinophilic asthma is generally well controlled by corticosteroids that induce the apoptotic death of eosinophils via inhibition of the production of essential survival cytokines for these cells, such as IL-5, IL-3, and granulocyte macrophage colony-stimulating factor (GM-CSF) [7, 8]. Nevertheless, some patients with eosinophilic airway inflammation are refractory to corticosteroids, thus manifesting a severe, uncontrolled asthma featured by recurrent exacerbations. It is very likely that in these subjects with difficult-to-treat eosinophilic asthma a very intense activation of type-2 inflammatory pathways occurs, leading to an exaggerate overexpression of IL-5, which remarkably decreases eosinophil sensitivity to corticosteroids [9]. In fact, the proapoptotic effect exerted by corticosteroids on

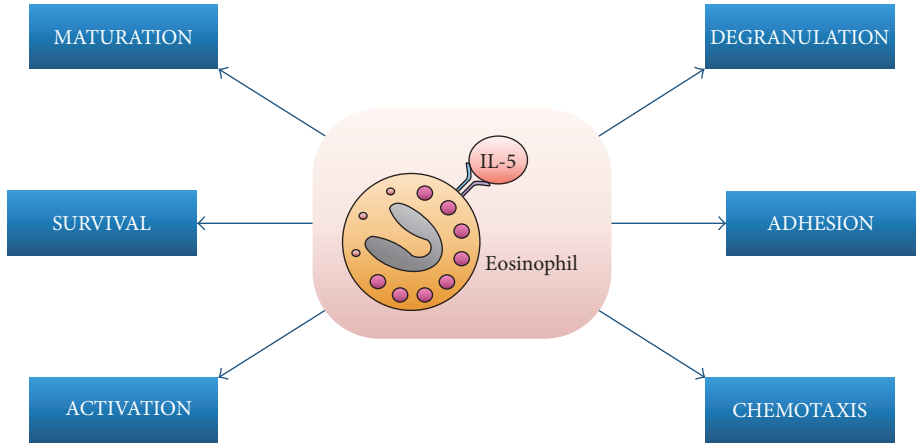

Figure 1: Main biological effects exerted by IL-5 on eosinophils.

eosinophils vanishes when these cells are subjected to the antiapoptotic action of high IL-5 levels [10].

These considerations thus strongly indicate that IL-5 is of paramount importance for the development, persistence, and amplification of eosinophilic asthma associated with a type-2 inflammatory/immune response. This implies that IL-5 and its receptor currently represent key molecules to be targeted by useful biological drugs for the treatment of severe eosinophilic asthma [11, 12]. Indeed, two humanized anti-IL-5 monoclonal antibodies (i.e., mepolizumab and reslizumab) have been designed, developed, and approved for add-on therapy of refractory eosinophilic asthma, whereas a third biologic drug (benralizumab) acts via a blockade of the IL-5 receptor [13–20]. These pharmacological options may thereby satisfy the unmet needs of patients with severe eosinophilic asthma who cannot achieve an adequate control of their disease, despite the use of high doses of therapeutic combinations of inhaled corticosteroids (ICS) and long-acting β_2-adrenergic agonists (LABA), eventually integrated by the addition of other controller medications including inhaled anticholinergics, as well as oral corticosteroids and leukotriene inhibitors [21, 22].

Therefore, the aim of the present review article is to briefly outline the role of IL-5 in eosinophilic asthma and especially to discuss the mechanism of action and the clinical effects of the IL-5 receptor antagonist benralizumab.

2. The Pathobiologic Role of IL-5 in Eosinophilic Asthma

IL-5 is mainly produced by Th2 lymphocytes and ILC2 cells [27–31]. Th2 lymphocytes synthesize and release IL-5 when they are activated by antigen-presenting dendritic cells, in the presence of IL-4 acting via stimulation of the transcription factors STAT6 and GATA3, whilst ILC2 produce IL-5 upon activation mediated by airway epithelium-derived alarmins including IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which also activate GATA3 [32].

The main biological effects exerted by IL-5 on eosinophils are summarized in Figure 1. In the bone marrow, as well as in the airways of patients with allergic asthma, IL-5 induces eosinophil differentiation and maturation from CD34+ hematopoietic progenitor cells [33, 34]. Indeed, high amounts of IL-5, eosinophil progenitors, and mature eosinophils can be found in the induced sputum obtained from these subjects. Moreover, by synergistically cooperating with powerful chemoattractants for eosinophils such as eotaxins 1, 2, and 3, IL-5 significantly contributes to the recruitment of these cells into the airways of asthmatic patients [15]. In particular, IL-5 promotes the interaction of eosinophils with vascular endothelial cells and their subsequent extravasation by upregulating the expression of key adhesion molecules such as α_L integrin (CD11a), α_M integrin (CD11b), β_2 integrin (CD18), and P-selectin glycoprotein ligand-1 (PSGL-1/CD162) [35]. Recruitment of eosinophils into asthmatic airways is also favoured by IL-5-induced eosinophil adhesion to protein components of the extracellular matrix like periostin, whose expression is stimulated by type-2 inflammatory/immune response [35]. When released from ILC2 cells, IL-5 is also remarkably involved in the pathogenesis of late onset, nonallergic eosinophilic asthma [29, 30].

The biological actions performed by IL-5 on eosinophils are mediated by activation of the IL-5 receptor, a membrane protein consisting of two components, an α subunit (IL-5Rα), which is specific for IL-5, and a βc chain, which can bind not only IL-5 but also IL-3 and GM-CSF [36–40]. IL-5 binding to IL-5Rα induces the dimerization of α and βc receptor components and the subsequent activation of a complex signaling network including Janus kinase 2 (JAK2) with the associated signal transducers and activators of transcription (STAT) 1, 3, and 5, as well as several other kinases such as Lyn and Raf-1, mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K), and protein kinase C (PKC) [41–50]. Through these mechanisms, IL-5 stimulates eosinophil differentiation, survival, proliferation, adhesion, chemoattraction, and degranulation, the latter being responsible for the release of cytotoxic proteins such as eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil-derived neurotoxin (EDN),

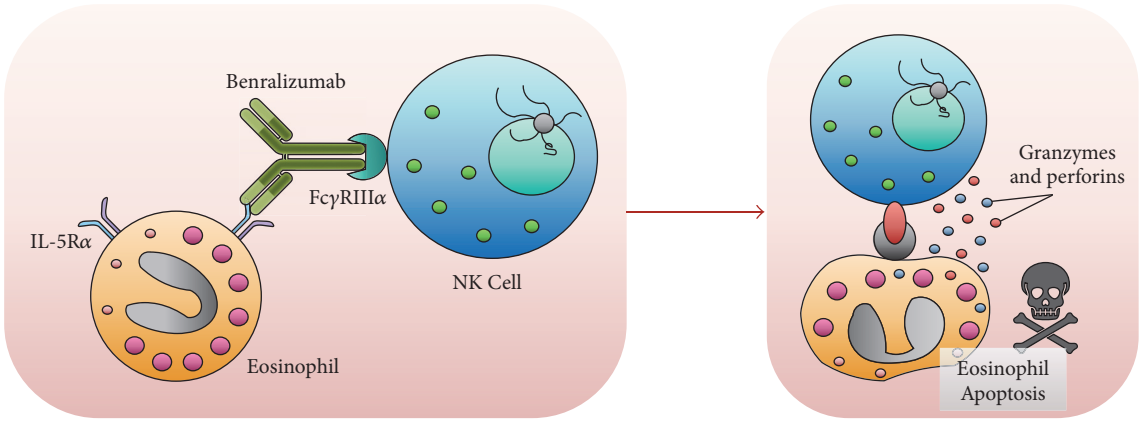

FIGURE 2: Mechanisms of action of benralizumab. Via its Fab fragments, the humanized monoclonal antibody benralizumab specifically binds to IL-5Rα, thereby preventing the interaction between IL-5 and its receptor. In addition, through its Fc constant region, benralizumab binds to the FcγIIIRa receptor expressed by natural killer cells, thus inducing eosinophil apoptosis operated by the release of proapoptotic proteins such as granzymes and perforins.

and eosinophil peroxidase (EP), which damage the airway epithelial cell layer. As a result of these cellular processes, high eosinophil counts can be found in bronchial specimens, induced sputum, and peripheral blood of patients with asthma triggered by type-2 inflammatory pathways [51–54].

3. Benralizumab: Mechanism of Action

Benralizumab (MEDI-563) was designed and produced by AstraZeneca/MedImmune by means of hybridoma technology [55–57]. This biologic drug is a humanized IgG1k monoclonal antibody generated in mouse and characterized by the specific feature of selectively binding to the amino acid residue isoleucine-61 included in domain 1 of human IL-5Rα. Via this linkage, benralizumab thus interacts with an extracellular IL-5Rα epitope that is situated in close proximity of the IL-5 binding site [58, 59]. This high-affinity interaction between benralizumab and IL-5Rα impedes IL-5 binding to its receptor and the consequent heterodimerization of α and βc subunits (Figure 2), thereby preventing the stimulation of the intricate signaling mechanisms coupled to IL-5 receptor activation.

Besides linking IL-5Rα through its Fab regions, benralizumab also binds via the constant Fc fragment to the FcγRIIIa receptor, located on cell membrane of natural killer (NK) cells (Figure 2) [60, 61]. In this regard, it is worth noticing that benralizumab was developed in Chinese hamster ovary cells not expressing the α-1,6-fucosyltransferase enzyme. As a consequence, lack of the fucose molecule in the sugar component of the CH2 domain of the constant segment of the monoclonal antibody is responsible for a remarkable enhancement (5 to 50 times) of benralizumab affinity for the FcγRIIIa receptor of NK cells [59]. In particular, with regard to the original fucosylated antibody, afucosylation makes benralizumab capable of inducing a ≥1000-fold amplification of the apoptotic mechanism named antibody-dependent cell-mediated cytotoxicity (ADCC). Indeed, benralizumab is a potent inducer of eosinophil apoptosis operated by NK cells through the release of the proapoptotic proteins perforin and granzyme B (Figure 2) [59]. Afucosylation-dependent ADCC has also been demonstrated by benralizumab-induced increase in eosinophil staining with annexin V, a well-known biomarker of apoptosis [59].

It can thus be argued that benralizumab is capable of killing eosinophils via a dual mechanism: the blockade of IL-5-mediated survival of these cells and the enhancement of eosinophil apoptosis induced by activation of the FcγRIIIa receptor of NK cells (Figure 2). By acting via such very powerful modalities, benralizumab rapidly and effectively depletes eosinophils in patients with asthma, thereby drastically reducing cell counts in both airways and peripheral blood [27].

4. Benralizumab: Clinical Trials in Asthma

In an early phase 1 study, Busse et al. evaluated the pharmacokinetics and pharmacodynamics of benralizumab by measuring eosinophil counts in peripheral blood throughout 12 weeks after intravenous injection to subjects with mild asthma of single doses ranging from 0.0003 to 3 mg/kg [62]. Benralizumab induced a dose-dependent and long-lasting depletion of blood eosinophils, which persisted at least 8 or 12 weeks at dosages of 0.03 to 0.1 or 0.3 to 3 mg/kg, respectively, whilst no significant effect was elicited by doses included within a range of 0.0003–0.003 mg/kg. Utilized at dosages ranging from 0.03 mg/kg to 3 mg/kg, the mean maximum concentration (1–82 μg/mL) of benralizumab resulted to be dose-proportional. The mean distribution volume (52–93 mL/kg) of the drug was greater than the plasma volume, thus suggesting that benralizumab probably binds to circulating cells that express IL-5Rα, and can also moderately penetrate into extravascular tissues. Consistent with the pharmacokinetic profile of human IgG antibodies, benralizumab had a mean elimination half-life of 2-3 weeks.

Another phase 1 trial was later carried out by Laviolette et al. who studied 26 asthmatics enrolled because of

their ascertained airway hyperresponsiveness or reversible airflow limitation [63]. 8 participants, treated with a single intravenous injection of 1 mg/kg of benralizumab, were comparatively evaluated with 5 patients randomized to receive placebo. Moreover, benralizumab was subcutaneously administered every month for 3 months at dosages of 100 and 200 mg to 4 and 5 subjects, respectively, who were compared to 4 patients treated with placebo. In order to assess airway eosinophilia, bronchial mucosal and submucosal samples were obtained via bronchoscopy at baseline and 28 days after the conclusion of therapy. In addition, eosinophil counts were also performed in peripheral blood. In some patients, eosinophils were also measured in induced sputum and bone marrow specimens. Given either intravenously or subcutaneously, benralizumab effectively depleted eosinophils in both peripheral blood and bone marrow. When compared to placebo, benralizumab also lowered eosinophil counts in induced sputum and bronchial samples, though such reductions did not result to be statistically significant. Hence, these findings indicate that, differently from bone marrow cells, which were completely sensitive to benralizumab, bronchial eosinophils did not completely respond to this drug. Therefore, it could be inferred that airway eosinophils, because of their more advanced stage throughout the maturation process, are less responsive to biological therapies targeting either IL-5 or its receptor [64]. Benralizumab was also capable of significantly decreasing the numbers of IL-5 receptor expressing blood basophils [63], which have been shown to be increased in bronchial walls of allergic asthmatic patients [65, 66].

Subsequently, a phase 2a study was conducted by Park et al. in asthmatic subjects with ≥ 2% sputum eosinophils or FeNO ≥ 50 ppb (parts per billion), treated with medium/high doses of ICS/LABA combinations, who had manifested 2–6 exacerbations of asthma during the previous 12 months [67]. In particular, 27 patients were randomly assigned to receive placebo, whereas benralizumab was administered via the subcutaneous route at doses of 2 mg, 20 mg, and 100 mg to 27, 26, and 26 subjects, respectively. Treatments with either drug or placebo were carried out at baseline and after 4, 8, 16, 24, 32, and 40 weeks. When compared to placebo, at the 52nd week, benralizumab decreased the annual asthma exacerbation rates by 33, 45, or 36% when utilized at dosages of 2, 20, or 100 mg, respectively. Moreover, lung function was improved during this study by all dosages of benralizumab, which enhanced forced expiratory volume in one second (FEV_1), thus inducing the highest increase at the 52nd week after administration of the 100 mg dosage, especially in patients having blood eosinophil levels ≥ 300 cells/μL (mean FEV_1 increase: 28.1%). Furthermore, benralizumab markedly reduced blood eosinophil counts. At all dosages, benralizumab was well tolerated and its safety profile did not result to be significantly different with respect to placebo.

Castro et al. performed a phase 2b study, which was completed by 324 eosinophilic and 282 noneosinophilic patients with uncontrolled asthma, treated with medium-high doses of ICS/LABA combinations, who during the previous 12 months had manifested 2–6 disease exacerbations [68]. Eosinophilic patients were randomly subdivided into four groups, assigned to receive placebo ($n = 80$) or benralizumab at dosages of 2 ($n = 81$), 20 ($n = 81$), or 100 mg ($n = 82$), respectively. Noneosinophilic subjects were randomized to receive either placebo ($n = 142$) or benralizumab ($n = 140$) at a dose of 100 mg, respectively. Both benralizumab and placebo were injected subcutaneously every 4 weeks with regard to the first 3 administrations (1st, 4th, and 8th weeks) and subsequently every 8 weeks (16th, 24th, 32nd, and 40th weeks). When compared with placebo, at the 52nd week, benralizumab decreased the annual rates of asthma exacerbations in eosinophilic participants who had been treated with drug dosages of 100 mg but not 20 mg or 2 mg. Benralizumab lowered asthma exacerbations to a greater extent in patients with blood eosinophil numbers ≥ 300 cells/μL. In these subjects, exacerbations were decreased by both drug doses of 100 mg and 20 mg. In noneosinophilic asthmatics, at the dosage of 100 mg, benralizumab did not reduce the annual exacerbation rate. In eosinophilic patients, all dosages of benralizumab lowered blood eosinophil numbers, improved symptom control, and enhanced FEV_1 values. With respect to placebo; benralizumab caused a slightly higher number of mild-to-moderate adverse reactions; nasopharyngitis and local irritations at injection sites were the most frequently observed events.

Another phase 2 study was performed by Nowak et al. who assessed the impact on hospitalization for acute asthma and/or on recurrence of disease exacerbations of a single intravenous administration of benralizumab prescribed as add-on biological therapy on discharge from emergency department [69]. The 108 participants who completed the study were subdivided into 3 groups of 36 patients, randomly assigned to receive either placebo or benralizumab at a dosage of 0.3 mg/kg or 1.0 mg/kg, respectively. The recruited asthmatic subjects were selected on the basis of specific features referring to the previous year, including at least one asthma exacerbation needing an urgent care visit, as well as an access to emergency department for acute asthma, only partially responsive to standard therapy. When compared with placebo, after 12 weeks of treatment, benralizumab induced significant decreases of 49% and 60% in the rates of asthma exacerbations and exacerbations requiring hospitalization, respectively. These effects were associated with drastic reductions in blood eosinophil counts and also in serum concentrations of the eosinophilic cytotoxic proteins ECP and EDN. Such findings were reported with regard to administration of both benralizumab dosages (0.3 mg/kg and 1.0 mg/kg). Benralizumab was characterized by a good safety pattern. In fact, only mild-to-moderate and self-limiting adverse reactions occurred, such as cough, bronchitis, fever, headache, muscle spasms, dizziness, hyperhidrosis, and anxiety. After 12 weeks of treatment, anti-benralizumab antibodies were found in 6 patients, but no clinical consequence was reported. In addition to Nowak et al., also Pham et al. showed that benralizumab significantly reduced the serum concentrations of ECP and EDN [70]. Therefore, such results further validate the concept that benralizumab can be able not only to decrease blood eosinophil counts but also to inhibit eosinophil degranulation and the consequent release of cytotoxic proteins.

Table 1: Benralizumab: summary of the main phase 3 clinical trials.

Authors and trial name	Duration	Number of patients	Main results
Bleecker et al. (2016) [23], SIROCCO	48 weeks	1205	Fewer asthma exacerbations, higher FEV_1
FitzGerald et al. (2016) [24], CALIMA	56 weeks	1306	Fewer asthma exacerbations, higher FEV_1
Nair et al. (2017) [25], ZONDA	28 weeks	220	Lower intake of oral corticosteroids, fewer asthma exacerbations
Ferguson et al. (2017) [26], BISE	12 weeks	211	Smaller numbers of blood eosinophils

In addition to phase 1 and phase 2 trials, many phase 3 studies have recently led to the approval of benralizumab by US FDA for the add-on biological therapy of severe eosinophilic asthma. In this regard, six phase 3 trials (SIROCCO, CALIMA, ZONDA, BORA, BISE, and GREGALE) are included within the so-called WINDWARD program [19]. The main phase 3 trials are summarized in Table 1.

In the SIROCCO study, Bleecker et al. randomized 1205 asthmatics, receiving high doses of ICS/LABA, to be treated for 48 weeks with one of the following add-on subcutaneous therapies: 407 subjects received placebo; 400 participants were treated with 30 mg of benralizumab every four weeks (Q4W); 398 patients were assigned to receive 30 mg of benralizumab every eight weeks (Q8W) [23]. When compared with placebo, after 48 weeks of treatment, benralizumab lowered the annual rates of asthma exacerbations by 45% and 51% in participants belonging to Q4W and Q8W groups with ≥300 blood eosinophils/μL, respectively. It is also worth noticing that the annual rate of asthma exacerbations diminished by 17–30% in patients with <300 blood eosinophils/μL. Furthermore, with respect to placebo, at the 48th week, both regimens of benralizumab significantly increased prebronchodilator FEV_1, with mean changes above baseline values of 106 mL and 159 mL in Q4W and Q8W dosage schemes, respectively. When compared to placebo, benralizumab induced a better control of asthma symptoms only in the Q8W group. The most commonly reported adverse reactions were nasopharyngitis detected in 12% of subjects receiving either placebo or benralizumab and worsening of asthma observed in 13% of patients receiving benralizumab and in 19% of participants treated with placebo, respectively.

FitzGerald et al. carried out the CALIMA trial, another study that recruited patients with asthma not adequately controlled by medium-to-high doses of ICS/LABA combinations who manifested two or more disease exacerbations during the previous 12 months [24]. Similar to the SIROCCO study, throughout 56 weeks, 440 participants received placebo, whilst 425 and 441 patients were treated subcutaneously with 30 mg of benralizumab every four (Q4W) or eight weeks (Q8W), respectively. When compared with placebo, Q4W and Q8W dosage schemes significantly lowered the annual rates of asthma exacerbations by 36% and 28%, respectively. Furthermore, in subjects having ≥300 blood eosinophils/μL, Q4W and Q8W regimens induced mean improvements in prebronchodilator FEV_1 of 125 mL and 116 mL, respectively. Moreover, both dosages of benralizumab drastically decreased blood eosinophil numbers, whereas only the Q8W schedule elicited a better control of asthma symptoms with respect to placebo. Nasopharyngitis (21% in the Q4W regimen, 18% in the Q8W arm, and 21% in the placebo group, resp.) and asthma worsening (14% in the Q4W cohort, 11% in the Q8W regimen, and 15% in the placebo group, resp.) were the most frequent adverse events.

Pooled results from SIROCCO and CALIMA trials have been recently analyzed by Chipps et al. who showed that benralizumab effectively decreased asthma exacerbations and also improved both lung function and quality of life in patients with eosinophilic asthma, regardless of serum IgE levels and atopic status [71]. Therefore, these findings indicate that benralizumab can be very useful for the management of severe eosinophilic asthma, associated or not associated with an allergic trait.

Nair et al. conducted the ZONDA study with the aim of evaluating, in severe uncontrolled asthmatics, the eventual ability of benralizumab to decrease the consumption of oral corticosteroids [25]. In particular, 220 patients were randomly assigned to a subcutaneous treatment, performed every 4 (Q4W) or 8 (Q8W) weeks for 28 weeks, with either placebo or benralizumab 30 mg. At the beginning of the trial, all participants were on maintenance treatment with oral glucocorticoids (median dose: 10 mg/day; range: 7.5–40 mg/day), whose median daily dosage resulted to be reduced at the end of the study by 75% in both groups of patients receiving benralizumab and by 25% in subjects treated with placebo, respectively ($p < 0.001$) [25]. Moreover, with respect to placebo, benralizumab lowered the annual rates of asthma exacerbations by 55% and 70% in Q4W and Q8W subgroups, respectively. Benralizumab and placebo did not differ with regard to their effects on FEV_1. Finally, benralizumab and placebo were characterized by similar profiles of safety and tolerability; with both treatments, the most frequent adverse events were asthma worsening, nasopharyngitis, and bronchitis.

The aim of the BORA trial (ClinicalTrials.gov Identifier: NCT02258542) is to evaluate the safety and tolerability pattern of benralizumab in subjects with asthma already enrolled in either SIROCCO, CALIMA, or ZONDA.

The BISE study was carried out by Ferguson et al. in patients with mild-to-moderate persistent asthma, receiving low-to-medium ICS dosages, who were randomly treated via the subcutaneous route, every 4 weeks for 12 weeks, with either placebo or 30 mg of benralizumab [26]. With respect to placebo, at the 12th week, benralizumab induced a prebronchodilator FEV_1 increase of 80 mL. FEV_1 changes did not result to be different with regard to baseline blood eosinophil counts higher or lower than 300/μL. Furthermore, differently from placebo, benralizumab completely and persistently (up to the 20th week) depleted blood eosinophils. Patient groups

receiving benralizumab or placebo experienced similar incidences of adverse reactions, mostly consisting of upper respiratory tract infections, nasopharyngitis, headache, and asthma worsening.

The GREGALE open label trial has recently demonstrated that the majority of severe asthmatic patients and their family members, recruited in the study, were able at home to efficiently use an accessorized prefilled syringe, prepared for subcutaneous injections of benralizumab 30 mg [19].

A meta-analysis referring to 1951 subjects with eosinophilic asthma, enrolled in several different phase 1, 2, and 3 randomized controlled trials, demonstrated that, when compared to placebo, benralizumab induced significant score improvements of asthma control questionnaire-6 (ACQ-6) and asthma quality of life questionnaire (AQLQ) and enhanced FEV_1 and also decreased the annual rate of disease exacerbations [72]. In addition, this meta-analysis showed that the dosage schedule of benralizumab 30 mg every 8 weeks produced better results than the same dose injected every 4 weeks. Furthermore, benralizumab and placebo induced similar patterns of adverse effects.

Besides the WINDWARD program, other ongoing phase 3 trials are ANDHI, MIRACLE, and SOLANA [19]. The ANDHI study (ClinicalTrials.gov Identifier: NCT03170271) aims to assess, in patients with severe eosinophilic asthma, the effects of benralizumab on asthma exacerbations, lung function, and quality of life. In addition, some subjects enrolled in this trial will be also studied with regard to the effects of benralizumab on relevant comorbidities of asthma, such as nasal polyposis and chronic rhinosinusitis. The MIRACLE study (ClinicalTrials.gov Identifier: NCT03186209) is another trial designed to primarily investigate, in severe asthmatics receiving medium-to-high doses of ICS/LABA medications, the eventual ability of benralizumab to lower the annualized rate of asthma exacerbations. SOLANA (ClinicalTrials.gov Identifier: NCT02869438) is currently assessing, in patients with severe eosinophilic asthma, the impact of benralizumab on symptom score, quality of life, lung function, and blood eosinophils.

Finally, a very recent study performed by Sehmi et al. on 18 patients with severe eosinophilic and corticosteroid-dependent asthma showed that 30 mg of benralizumab, administered subcutaneously every 4 or 8 weeks, when compared to placebo, significantly decreased the counts of mature eosinophils in both blood and induced sputum [73]. In blood, benralizumab also significantly reduced the number of eosinophil progenitors. A similar result was also detected in induced sputum, where, however, this effect of benralizumab did not reach the threshold of statistical significance, probably because of the small number of matched data sets [73]. Moreover, in blood, benralizumab significantly lowered the number of ILC2 cells expressing IL-5Rα, and a similar effect was also observed in induced sputum, where, however, only a trend, and not a significant difference, was found. Serum EDN concentrations were also significantly diminished by benralizumab. In addition, benralizumab significantly increased the levels of granzyme B and interferon-γ in cell-free sputum supernatants. Therefore, the latter findings suggest that benralizumab was able to stimulate the activity of NK cells. All these biological effects of benralizumab were paralleled by relevant clinical and functional improvements, including a decrease in the maintenance dosage of oral corticosteroids, a better asthma control, and an increased ratio of prebronchodilator FEV_1 to FVC (forced vital capacity).

5. Conclusions

It is currently clear that, given the pivotal functions exerted by IL-5 in the induction, maintenance, and amplification of airway eosinophilia driven by type-2 inflammation, such a cytokine and its receptor represent key molecules to be targeted by monoclonal antibodies with therapeutic properties of add-on biological treatments for severe eosinophilic asthma. In particular, because of its very effective action as IL-5Rα antagonist, benralizumab has been shown to be capable of significantly inhibiting eosinophil differentiation in the bone marrow, as well as eosinophilic infiltration of airways. These eosinopenic effects are further potentiated by ADCC-mediated eosinophil apoptosis, operated by NK cells, and stimulated by benralizumab. At clinical and functional levels, such a dual mechanism of action of benralizumab translates into relevant improvements, including a significant decrease of asthma exacerbations, a better symptom control, a marked sparing effect on the intake of oral corticosteroids, and an important attenuation of airflow limitation [74–76]. All these features, associated with a very good safety and tolerability profile, make benralizumab a valuable therapeutic option for add-on biological treatment of severe eosinophilic asthma.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Exosomes in Severe Asthma: Update in Their Roles and Potential in Therapy

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Exosomes are nanosized vesicles and have recently been recognized as important players in cell-to-cell communication. Exosomes contain different mediators such as proteins, nucleic acids (DNA, mRNA, miRNAs, and other ncRNAs), and lipid mediators and can shuttle their exosomal content to both neighboring and distal cells. Exosomes are very effective in orchestrating immune responses in the airways and all cell types can contribute to the systemic exosome pool. Intracellular communication between the broad range of cell types within the lung is crucial in disease emphasizing the importance of exosomes. In asthma, exosomes affect the inflammatory microenvironment which ultimately determines the development or alleviation of the pathological symptoms. Recent studies in this area have provided insight into the underlying mechanisms of disease and led to interest in using exosomes as potential novel therapeutic agents.

1. Introduction

Asthma is a heterogeneous syndrome involving inflammation and obstruction of the airways that affects 300 million people worldwide [1, 2]. Limited knowledge of the disease mechanisms is the greatest obstacle to the development of novel treatments. Although two forms of asthma have been traditionally defined in the clinic (T2 and non-T2), this ignores the broad range of phenotypes that have been described and the underlying pathophysiology of these phenotypes. As a result, asthma is increasingly recognized as a syndrome rather than a single disease [3, 4]. The goal of asthma research is to link asthma classification based on phenotypes with pathophysiological mechanism and thereby define asthma endotypes which will predict drug efficacy [4]. Several asthma phenotypes have been described such as allergic bronchopulmonary mycosis and severe late-onset hypereosinophilic asthma [4, 5]; however, a small group of

patients have asthma that is uncontrolled or only partially controlled despite intensive treatment [6]. This form of asthma is commonly referred to as severe asthma [7] which is often associated with serious morbidity and even mortality [6].

The emergence of biomarkers such as blood eosinophils linked with T2-asthma targeted biologic therapies opens new hopes for patients with severe asthma. However, further research is required to understand the mechanisms underlying pathophysiology of severe non-T2 asthma and to define the optimal biological treatment. In addition to this it is important to have readily accessible biomarkers that define patient subsets to ensure that the correct drug is given to the right patient at the right time. This is essential for the patients' perspective and for the healthcare provider where the current blunt measures such as blood eosinophils do not distinguish differences in underlying pathophysiological processes.

Exosomes are small vesicles (30–100 nm in diameter) that enable cell-to-cell communication by shuttling different molecules such as nucleic acids (DNA, mRNA, and micro (mi)RNAs), lipids, proteins such as heat shock 70-kDa protein (HSP)70, and specific cell surface markers reflecting the exosome cell of origin. These would include CD9, CD63, and CD81 if the exosome was endosomal in origin [8]. Exosomes can, therefore, significantly affect target cell function resulting in the development of a pathological state [9].

Exosomes have been most extensively studied in association with the pathogenesis of diverse diseases, such as cancer [10, 11] and infectious disease [12–14] as well as in asthma [15]. Exosome biology has provided us with fundamental insights into the mechanisms of cellular crosstalk in asthma and may also act as important biomarkers of the disease. In this review we summarize recent advances regarding the roles of exosomes in the pathogenesis of severe asthma and discuss their potential as biomarkers for targeted treatments.

2. Asthma Pathogenesis

Asthma is a complex disease whose underlying pathophysiology is not completely understood [16]. As a chronic inflammatory airway disease, asthma involves many cells from the innate and adaptive immune systems which act on airway epithelial cells to trigger bronchial hyperreactivity and airway remodeling in response to environmental stimuli such as allergens, infections, or air pollutants [3, 17]. The main features of allergic asthma are increases in the numbers and activity of airway mast cells and eosinophils which are due to the pathophysiological effects of proinflammatory cytokines such as interleukin- (IL-) 4, IL-5, and IL-13 released by activated CD4+ T-cells (Th2 cells) in response to environmental allergens [3]. In addition to lymphocytes and plasma cells, a large number of eosinophils and neutrophils are observed in the bronchial tissues and mucus of asthmatic airways [18].

During an asthma attack, airway provocation with allergens triggers a rapid decrease in bronchial airflow with an early immunoglobulin E- (IgE-) mediated reaction that may be followed by a late-phase IgE-mediated decrease in bronchial airflow for 4–8 hours [19]. Based on our understanding of the pathophysiology of allergic asthma, activated CD4 T-lymphocytes recruit leukocytes to the airway from the bloodstream and the presence of these stimulated leukocytes results in the secretion of inflammatory mediators from eosinophils, mast cells, and lymphocytes within the airway. The expression of Th2 cytokines from activated T-lymphocytes also directs the switch from IgM to IgE antibody production [20]. Mast cell activation and degranulation are triggered following cross-linking of the membrane bound high affinity IgE receptor (FcεRI) on mast cells which causes them to release inflammatory lipid mediators such as histamine and leukotrienes (LTs). In addition, IL-5 directs the recruitment of eosinophils from the bone marrow to the site of airway inflammation [21, 22]. Chronic inflammation in the asthmatic airway leads to repeated cycles of tissue injury and repair which results in structural alterations and remodeling of the airways over time [23, 24] (Figure 1).

3. Exosome Properties and Function

Exosomes are small 30–100 nm membrane-enclosed vesicles. They were discovered in 1983 and initially were described as small vesicles that bud from reticulocytes during their maturation and thought to function as the cell's "garbage bin" [8]. Further studies indicated that exosomes are released from most mammalian cells and are found in nearly all biological fluids [25]. Later studies determined the biological function of exosomes [26, 27] and highlighted their involvement in many pathological conditions such as cancer and neurodegenerative and infectious diseases as well as in immunemodulatory processes [28, 29]. The watershed moment in the study of exosomes came in 2007 with the finding that exosomes contained more than 1200 mRNAs which were translated into proteins following delivery to recipient cells [29, 30]. The crucial importance of exosomes, therefore, lies in their capacity to shuttle information between cells and influence the function of recipient cells [12]. Exosomes have also recently been implicated in cell homeostasis and the removal of unwanted molecules [31].

Exosome-derived signaling molecules include proteins, lipids, nucleic acids, and miRNAs whose packaging together gives an advantage of simultaneous delivery of multiple components to target cells [32]. An important feature of exosomes is that they are highly stable in biological fluids [33]. In addition, their content and composition resemble their cell of origin and these may change according to the physiological or pathological conditions the cell is exposed to [34]. Exosomes may contain many bioactive agents including prostaglandins and LTs, lipids, transmembrane receptors such as integrins $\beta 1$ and $\beta 2$, costimulatory molecules, membrane-localized classes I and II major histocompatibility complexes (MHC), signal transduction proteins, and nucleic acids (mRNA and miRNAs) [12] (Figure 2).

Extensive investigations have elucidated the role of exosomes in intercellular communication and the regulation of physiological functions and homeostasis as well as their contribution to various pathological conditions [32]. It is within this context that we review the function of exosomes in

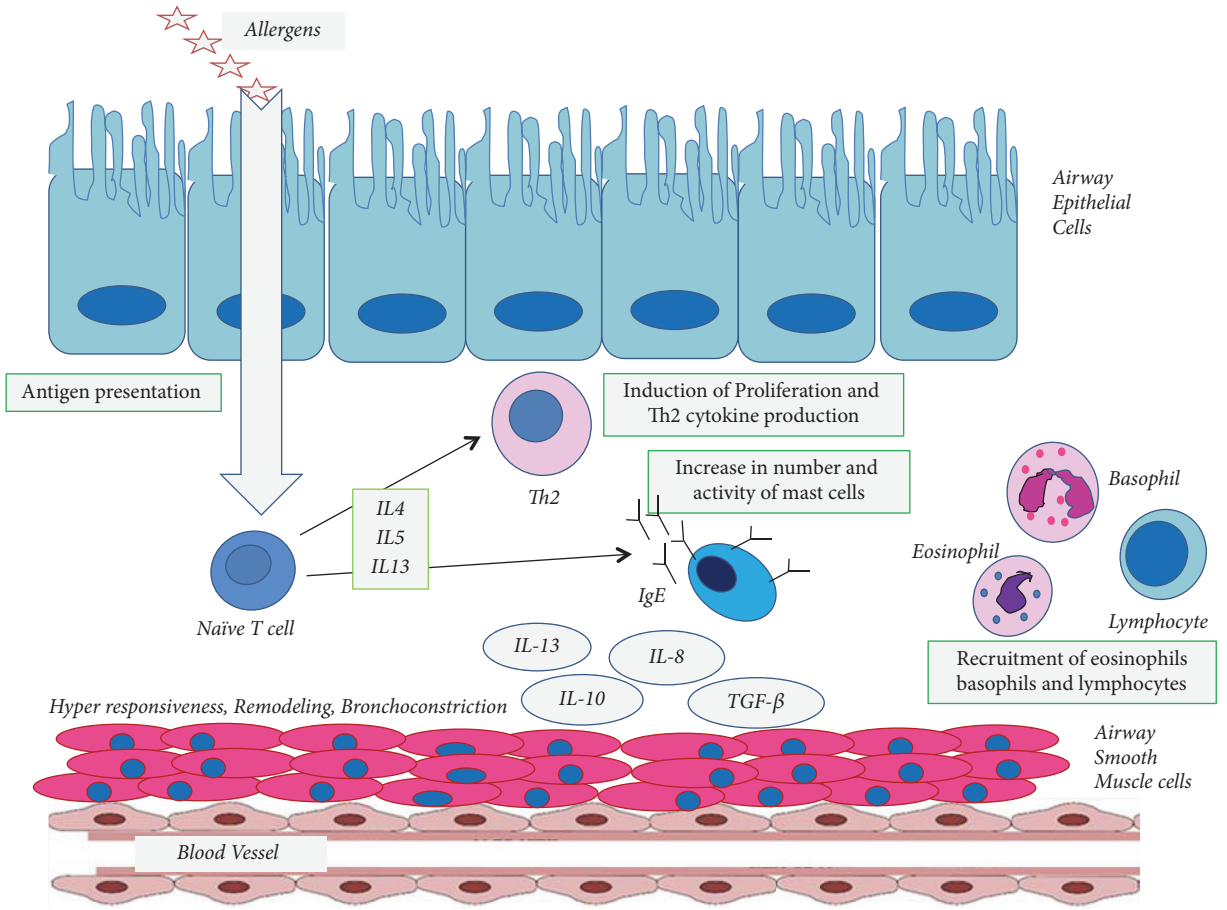

Figure 1: *The pathogenicity of asthma*. The entry of allergens into the airway triggers the Th2 response through the antigen presenting cells and induce the differentiation of naïve CD4+ T-cells into CD4+ Th2 cells in the presence of IL-4, IL-5, and IL-13. Activated CD4 T-lymphocytes recruit leukocytes to the airway from the bloodstream which will follow with the secretion of inflammatory mediators from eosinophils, mast cells, and lymphocytes within the airway. The expression of Th2 cytokines directs the switch from IgM to IgE antibody production. Mast cell activation and degranulation are triggered following cross-linking of the membrane bound high affinity IgE receptor on mast cells. Chronic inflammation in the asthmatic airway leads to repeated cycles of tissue injury and repair which results in structural alterations and remodeling of the airways over time.

the development of asthma with particular reference to severe disease.

4. Exosomes in Severe Asthma

The lung is a complex organ composed of a wide range of immune and structural cells within the parenchyma and airway [35]. For optimal functioning, cell-cell communication is essential and so exosomes are expected to play crucial role in lung biology and function [36]. In relation to the pathobiology of asthma, exosomes are released from the key cells implicated in disease such as mast cells, eosinophils, dendritic cells (DCs), T-cells, and bronchial epithelial cells. These in turn can trigger the activation, or repression, of other asthma-associated cells and enhance allergic responses [37].

DC-derived exosomes have costimulatory molecules on their surfaces that can activate allergen-specific Th2 cells [33, 38]. In addition, eosinophil-derived exosomes have important roles in the modulation of asthma and their numbers are increased in asthmatic patients [39, 40]. Analysis of exosomal miRNAs in patients with severe asthma compared with healthy subjects showed an altered miRNA content. The dysregulated miRNAs were involved in pathways related to airway integrity as well as being correlated with some clinical features such as eosinophil count or FEV1 [41]. In a separate study, the differential exosomal miRNAs profile in SA patients were associated with TGF-ß signaling pathway, the ErbB signaling pathway, and focal adhesion [42].

BAL exosomes from asthmatic patients express the epithelial marker mucin 1 on their surface indicating that they are derived from bronchial epithelial cells [43]. They were able to induce the production of CXCL-8 and LT C4 in target bronchial epithelial cells [44]. Whether this is a natural autocrine effect of these exosomes or whether other cells are the physiological target cell is unknown but BAL exosomal miRNAs from asthmatics were involved in IL-13-mediated events [45]. In a feedback manner, IL-13 promotes exosome production by airway epithelial cells and these exosomes subsequently enhance the proliferation of undifferentiated lung macrophages [44]. Thus, both structural and effector cells

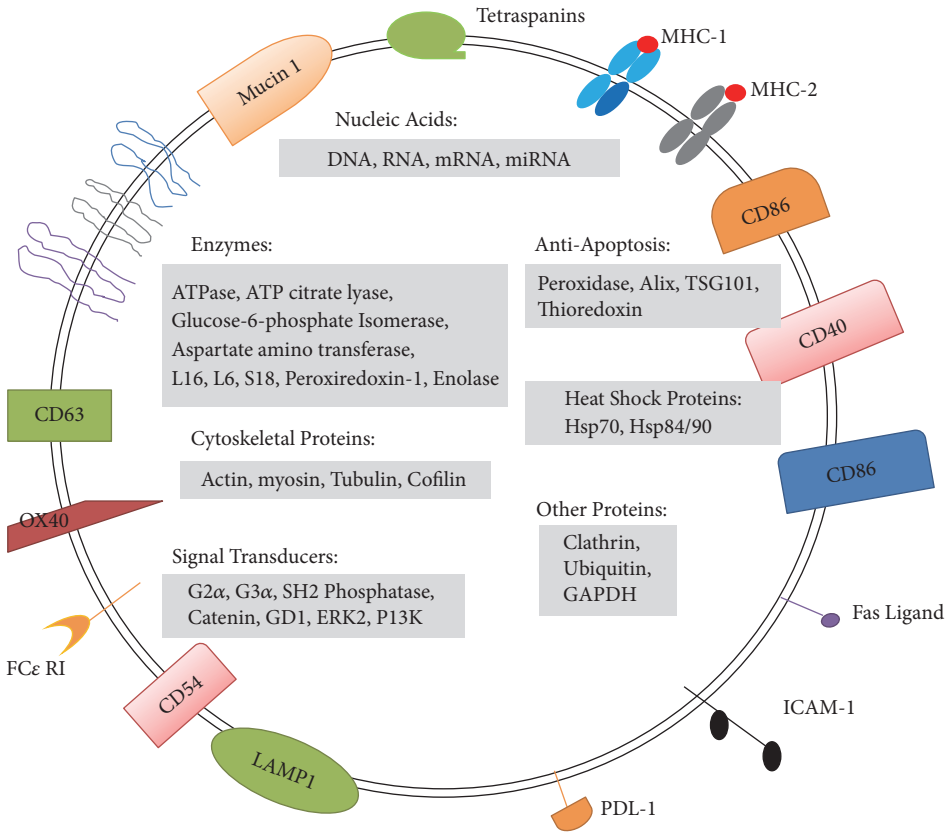

FIGURE 2: *Exosomes are small membrane-enclosed vesicles* containing mRNA and miRNA, lipids, and a vast array of different proteins depending on their cell of origin. Generally exosomes are enriched in some of generic proteins such as proteins involved in MVB formation, tetraspanins, and membrane transports as well as a number of cytosolic proteins. In addition some compounds associated with specific pathological condition have been identified in exosomes.

produce exosomes that modulate the chronic inflammatory processes involved in asthma [15].

4.1. Exosomes from Immune Effector Cells. Inflammation is the main pathogenic driver in asthma. Exosomes can promote inflammation via regulating the function of immune cells at the level of their recruitment, activation, or differentiation. A broad range of cells in lung are involved in asthmatic inflammation including airway epithelial cells [46–48], eosinophils [39, 49], lymphocytes [32, 46, 50], macrophages [46, 51], and DCs [48].

Eosinophils are multifunctional granulocytes that have an important role in both allergy and asthma due to their production, storage, and release of a range of inflammatory mediators. These include chemokines, lipid mediators, and cytotoxic granule proteins such as major basic protein (MBP), eosinophil peroxidase (EPX), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN), which together result in several key features of asthma.

Eosinophils from asthma patients release a greater number of exosomes in comparison with those released from cells of healthy subjects. These exosomes contain the main eosinophilic proteins such as EPO, MBP, and ECP and may, therefore, play a similar role in driving the progression of asthma as their parent cell [39]. Eosinophil-derived exosomes isolated from asthmatics may have both autocrine and paracrine functions as they increase in the production of chemokines, reactive oxygen species (ROS), and nitric oxide (NO) from target eosinophils as well as enhancing eosinophil migration by upregulating the expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and integrin $\alpha 2$ [49] which is a critical step in asthma development [15].

Lymphocytes are key players in the inflammatory response in allergy and asthma. B-lymphocytes produce antigen specific immunoglobulins E (IgE) following Th2 cell activation and release of Th2 cytokines [52]. B-lymphocytes can also trigger an asthmatic response by acting as an antigen presetting cell (APC) without the involvement of IgE and T-lymphocytes [53]. In addition, B-lymphocytes are involved in the differentiation of naïve Th0-lymphocytes into Th1- or Th2-lymphocytes by releasing IFN-γ or IL-4, respectively [54]. Finally, IL-10-producing B-cells or Breg (B regulatory) downregulate inflammation in hyperresponsiveness airway and suppress allergic inflammation by recruitment of natural Treg (CD4+ CD25+ FoxP3+) cells to the lung [55].

B-cell-derived exosomes resemble their parent phenotype and carry MHC classes I and II and integrins $\beta 1$ and $\beta 2$ as well

as the costimulatory molecules CD40, CD80, and CD86. As a result, they can specifically present antigenic peptides to T-cells and induce T-cell responses [56]. B-cell exosomes also contain HSP70 which is important in DC maturation [57]. B-cell-derived exosomes can also modulate the proliferation and production of Th2 cytokines from T2 cells due to the presence of exosomal antigens such as birch peptide (Bet v1) to the same degree as observed upon direct contact between B- and T-cells. This highlights the important roles of B-cell-derived exosomes in asthmatic inflammation as they can bypass the need for direct cell-to-cell contact [56].

Like other immune cells, T-lymphocytes can release exosomes [58–60]. Cytotoxic CD8+ T-cells release granules containing cytolysis mediators [60]. However, the bioactivity and potential immune-regulatory effect of T-cell-derived exosomes is not clear [61, 62]. Exosome released by T-cells is a selective and highly regulated process since T-cell receptor (TCR) activation, but not stimulation with mitogenic signals such as phorbol esters, greatly increases exosome production [58].

Exosomes released by activated CD4+ T-cells suppress the cytotoxic responses and antitumor immunity by CD8+ T-lymphocytes. These activated T-cells release 5–100 nm saucer-shaped exosomes that contain many proteins including lysosomal-associated membrane protein 1 (LAMP-1) and lymphocyte function associated antigen-1 (LFA-1) as well as CD4+ T-cell markers such as CD4, TCR, CD25, and Fas ligand [63]. Recent studies emphasize the importance of lipids in mediating T-cell-derived exosome production and function. These exosomes are enriched in sphingomyelin and cholesterol [64] and ceramide, tetraspanins, and myelin and lymphocyte (MAL) protein are important in T-cell exosome biogenesis [61]. MAL is a 17 KDa hydrophobic proteolipid located in the endoplasmic reticulum of T-cells and is involved in T-cell signal transduction. MAL was initially thought to be expressed only in T-cells but later was found also in myelin-forming cells and in polarized epithelial cells where it has a role in the apical transport of secretory proteins [65]. Activated CD3+ T-cells also release biologically active exosomes. These exosomes together with IL-2 triggered the proliferation of autologous resting CD3+ T-cells and induced a distinct cytokine profile [63].

In addition, several studies have shown that exosomes originating from other cell types can modulate T-cell function and subsequently affect the allergic asthmatic response [66–68]. For example, exosomes originating from B-cells [15], DCs [32], and epithelial-derived BALF exosomes [43] trigger T2 cytokine production along with increased proliferation and activation.

Mast cells are key immune cells in the development of allergic reactions and Th2 responses [69]. Activation of mast cells leads to the release of bioactive mediators such as histamine, prostaglandins, and LTs which subsequently trigger the allergic response. Mast cells also contribute to the secretion of proinflammatory cytokines such as TNF-α and IL-13 which drive the innate and adaptive immune responses in asthma [70, 71].

Mast cells constitutively release exosomes which have downstream effects on other immune cell types. For example, mast cell-derived exosomes induce DCs to acquire costimulatory MHC class II, CD80, CD86, and CD40 molecules enabling them to have an antigen presenting capacity for T-cells [72]. These exosomes can also modulate the activation of B- and T-lymphocytes and stimulate the production of cytokines such as IL-2, IL-12, and IFN-γ by these cells [73].

Mast cell-derived exosomes can enable target cell signaling from cell surface receptors upon contact with immune effector cells. For example, mast cell-derived exosomes trigger IgE production by B-cells in the absence of T-cells through their CD40 surface ligand [74]. Moreover, exosomes originating from bone marrow-derived mast cells (BMMCs) contain CD63 and OX40L on their surface and so can ligate with OX40 on the surface of T-cells and induce T-cell proliferation and differentiation of naïve T-cells to Th2 cells. BMMC-derived exosomes modulate the airway inflammation and remodeling responses seen in murine models of allergic asthma [75]. Mast cell-derived exosomes carry FcεRI which can bind to free IgE. This can result in decreased serum levels of IgE and limit the effects of mast cell activation. This indicates the potential of mast cell-derived exosomes as a novel anti-IgE factor in controlling the pathogenesis of severe asthma [75].

Lastly, mast cell-derived exosomes can also modulate T-cell function by donation of their contents [76] and induce the secretion of proinflammatory cytokines by human airway smooth muscle cells (ASMCs) which leads to preservation of asthmatic features [77].

Basophiles are a population of basophilic leukocytes and are like mast cells in that they are granular and are involved in allergic immune responses [78]. Basophiles comprise 0.5–1% of circulating white blood cells; however, upon inflammatory or chemotactic stimuli they increase in number and are recruited to the site of infection, for example. As with mast cells, basophils modulate the immune response by affecting other immune effector cells. Basophils can induce the proliferation and survival of naïve B-cells and direct their differentiation into antibody-producing cells. The crosstalk between these cells can be mediated via direct cell-to-cell contact as well as through soluble mediators and exosomes [79]. It was known for a long time that basophils release granules that resemble exosomes [80]; however there is limited evidence of exosome production by basophils [78].

Dendritic cells are specialized effector cells in the immune system. Acting as antigen presenting cells (APC) they process and present antigens to T-cells as well as having the capacity to phagocytose dead cells and bacteria and thereby contribute to innate immunity [81, 82]. Exosomes derived from DCs resemble their parent's morphology by possessing MHC classes I and II molecules on their surface enabling them to stimulate T-cell responses [66] or they may be captured by other APCs to induce immune responses [66]. DC-derived exosomes can present allergens and trigger the induction of Th2 responses [83]. For example, exosomes released from DCs obtained from subjects allergic to cat dander induce IL-4 responses in peripheral blood mononuclear cells (PBMCs) [38].

DC-derived exosomes contain HLA-DR, MHC, CD86, and CD54 on their surface. The presence of the costimulatory

molecule CD86 indicates the potential of these exosomes to induce T-cell proliferation and differentiation whilst CD54 enables exosomes to interact with T-lymphocytes via LFA-1 [84]. These exosomes also contain enzymes that can convert LT A4 to other LTs such as LTB4 and LTC4 [84].

These exosomes also contribute to the recruitment and migration of granulocytes and leukocytes to the site of inflammation. This process is mediated by metabolites of arachidonic acid (5-keto eicosatetraenoic acid, KETE, and LTB4) that are produced following transfer of exosome-derived enzymes. These proinflammatory lipid metabolites are important in triggering asthma pathogenesis [84].

4.2. Exosomes from the Lung Structural Cells. Exosomes released from structural lung cells also contribute to fine-tuning of the immune response in asthma via managing intercellular communication [8]. Exosomes released by bronchial fibroblasts can be taken up by bronchial epithelial cells. Intriguingly, although the levels of transforming growth factor- (TGF-) β2 in exosomes derived from severe asthmatic fibroblasts were lower than that in exosomes derived from healthy subjects, fibroblast-derived exosomes from severe asthmatics induced increased proliferation of epithelial cells. The level of TGF-β2 in the fibroblast-derived exosomes was significantly related to the level in the cell of origin which controlled the exosome effect on bronchial epithelial cell proliferation. Thus, modulation of fibroblast TGF-β2 levels by overexpression or knockdown had concomitant effects on exosome levels of TGF-β2 and on epithelial cell proliferation [85].

The production of exosomes by lung cells and their protein content was higher in a mouse model of asthma. In this model IL-13 augmented the secretion of exosomes by lung epithelial cells and these exosomes enhanced the proliferation and differentiation of macrophages. Inhibition of exosome production by GW4869 alleviated the induction of asthmatic features in this model [44].

5. Exosomal miRNAs in Severe Asthma Pathogenesis

Exosomes as important mediators of cell communication can deliver miRNAs from one cell to a distinct target cell at a neighboring or distal site and subsequently affect the function of the target cell [86]. miRNAs modulate both innate and adoptive immune response with miR-21, miR-146a, and miR-155 being reported as key miRNAs in the asthmatic immune response [87]. Each miRNA can target hundreds of genes; so any changes in miRNAs level can influence many signaling pathways and have profound effects on disease pathogenesis [88].

In asthma, dysregulated miRNA expression has been observed in many cells and compartments including airway biopsies, lymphocytes, epithelial cells, and peripheral blood [89]. For example, in a murine model of asthma upregulation miR-21 was associated with altered IL-12 expression and a heightened Th2 response [90, 91]. Overexpression of miR-21 along with miR-126 was also detected in airway epithelial cells of asthmatic patients [92]. Other dysregulated miRNAs include miR-1248, miR-let7a, miR-570, miR-133a, and miR-328 which are decreased in plasma of asthmatic patient [93] whilst miR-221 was increased in ASMC from patients with severe asthma and regulated ASM proliferation and the secretion of proinflammatory mediators such as IL-6 [94].

Similarly, the exosomal miRNA content may also be altered in pathological conditions. For example, an analysis of BAL-derived exosomal miRNAs in asthma reveals the altered expression of 24 miRNAs in asthmatic patients compared to healthy subjects which are implicated in the regulation of IL-13-mediated functions [95]. In addition, CD8+ cells released exosome like vesicles that contain miR-150 and are coated with antigen specific antibody [87]. Internalization of these vesicles by the T-cells leads to antigen specific tolerance in mice [87].

Analysis of circulating exosomal miRNAs by next-generation sequencing demonstrated upregulation of miR-128, miR-140-3p, miR-196b-5p, and miR-486-5p in severe asthma patients in comparison to healthy subjects. These differentially expressed miRNAs were mostly involved in ErbB signaling pathway and focal adhesion [42]. In another study, the altered severe asthma exosomal miRNA content was associated with airway epithelial cell integrity and feature of asthma such as peripheral blood granulocyte counts [41].

6. The Therapeutic Potential of Exosomes in Asthma

Exosomes can regulate homeostasis and vital immune functions in the lung microenvironment. Exosomal contents have recently been suggested as potential diagnostic biomarkers in multiple diseases. In addition, as described above, exosomes can act as traps to prevent immune activation. Mast cell-derived exosomes possess FCεR1 on their surface which can bind free serum IgE and limit the effects of mast cell activation [75]. Furthermore, CD8+ cells release microvesicles that contain miR-150 which can suppress allergic contact dermatitis (ACD) and induce an antigen specific tolerance in mice [87].

Mesenchymal stem cells (MSC) release exosomes with the capacity to accelerate wound healing and lung tissue regeneration and this may be of use in alleviating airway remodeling in asthma [96]. These exosomes also have anti-apoptotic and anti-inflammatory properties indicating that they may be effective in other lung chronic inflammatory conditions [97]. Clinical trials using exosome-based therapy in acute respiratory distress syndrome (ARDS) are being conducted [98]. In an animal model of ARDS, exosomes derived from MSC reduce lung inflammation via induction of keratinocyte growth factor (KGF) expression in the injured alveolus and thereby improve the lung protein permeability [99].

Immunotherapy for non-small-cell lung cancer (NSCLC) using dexosomes is also undergoing clinical trials. Dexosomes are DC-derived exosomes that are loaded with tumor antigen [100]. The data to date indicate that exosome therapy is feasible and safe and may represent an alternative approach to traditional therapeutic methods in inflammatory diseases such as asthma. Further studies are required to examine the effect of exosomes on the different pathological features

associated with patients with distinct phenotypes of severe asthma.

7. Conclusion

In recent years, exosomes have emerged as an important area in biomedical research. Exosomes play a key role in local and distant intracellular communication and have been implicated as having a crucial role in the regulation of normal cellular function and increasingly in pathological conditions. These nanovesicles are also being increasingly recognized as potentially powerful tools for the prognosis, diagnosis, monitoring, and treatment of patients in many therapeutic areas.

Within the lung microenvironment cell-to-cell communication is of upmost importance. In asthma, exosomes can regulate immune and inflammatory responses in a beneficial and detrimental manner. The severity of asthma has been linked with distinct exosomal pools and/or content which have important roles in disease at least in primary cells and in in vivo models of disease. In addition, the unique constituents of exosomes indicate their potential as biomarkers or as novel therapeutic agents. However, there are still many unsolved problems in the area including the selectively packaging of exosomal content and the mechanisms involved in the precise delivery to target cells; these need to be elucidated.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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