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Review

Advances in patent applications related to allergen immunotherapy

Eduardo Santos da Silva^{1,2}, Carina Silva Pinheiro¹, Cristina Maria Quintella³, Fatima
Ferreira⁴, Luis Gustavo C. Pacheco¹, Neuza Maria Alcântara-Neves^{1,2*}

¹ Instituto de Ciências da Saúde – ICS, Universidade Federal da Bahia (UFBA), Bahia, Brazil

² Programa de Pós-Graduação em Biotecnologia da Rede Nordeste de Biotecnologia
(RENORBIO), Universidade Estadual do Ceará, Av. Paranjana, 1.700, Campus do Itaperi,
60740-000 Fortaleza/C

³ Instituto de Química, Universidade Federal da Bahia, Av. Adhemar de Barros, s/n., 40.170-
290, Campus de Ondina, Salvador, Bahia, Brazil

⁴ Department of Molecular Biology, University of Salzburg, Salzburg, Austria

***Corresponding author:**

Neuza Maria Alcântara-Neves

Laboratório de Alergia e Acarologia

Instituto de Ciências da Saúde

Universidade Federal da Bahia

Avenida Reitor Miguel Calmon, sem nº, Canela

CEP – 40110-100

Salvador, Bahia, Brazil

Tel: +55 71 3283 8932

E-mail: neuzalcantara@gmail.com

Abstract

Introduction: Allergies are among the most prevalent chronic diseases worldwide. Allergen-specific immunotherapy is used as an alternative treatment to pharmacotherapy. These immunotherapies are performed with crude extracts, which have disadvantages when compared to the new approaches, among them are recombinant proteins and hypoallergens. This review aims to assess immunotherapy for allergies through patent application analysis spanning recent decades.

Areas covered: Patents referring to allergen immunotherapies used in allergy treatment. Data were obtained from the Espacenet® website, using the Cooperative Patent Classification (CPC) system. Two-hundred-and-one patent applications were analysed, taking into consideration their classification by the type of technology and applicant.

Expert opinion: Allergen-specific immunotherapy represents the only potentially curative therapeutic intervention for the treatment of allergic diseases. The extract-based immunotherapy is being replaced by the use of recombinant allergens, highlighting the hypoallergenic forms, which have low IgE-binding while retaining T-cell reactivity. It is expected that the development of hypoallergens will expand the scope of allergen-specific immunotherapy, especially if associated with alternative systems for expression and delivery systems with future potential. Furthermore, these new developments will likely address the problem of long-term protocols in allergen-specific immunotherapy, thus allowing better patient adherence and compliance.

Keywords: Allergen-specific immunotherapy; allergy; DNA shuffling; hypoallergens; *in silico* mutagenesis; patent applications; recombinant peptides; technology assessment.

Abbreviation list

AIPN - Advanced industrial property network

CIPO - Canadian intellectual property office

CPC - Cooperative patent classification

DNA - Deoxyribonucleic Acid

EAPO - Eurasian patent organisation

ECLA - European classification system

EPO - European patent office

IgE - Immunoglobulin E

IL-13 - Interleukin 13

IL-4 - Interleukin 4

IL-5 - Interleukin 5

IL-9 - Interleukin 9

ILC2s - Group 2 innate lymphoid cells

IPC - International patent classification

JPO - Japan patent office

PCT - Patent cooperation treaty

SIT - Allergen-specific immunotherapy

SLIT - Sublingual immunotherapy

Tc2 - Cytotoxic T lymphocytes 2

T_H2 - Helper T lymphocytes 2

WIPO - World intellectual property organisation

Article highlights:

1. Comprehensive technological assessment review based on patent applications referring to allergen-specific immunotherapy for the treatment of allergies including new approaches in the area.
2. The dissemination of recombinant DNA technology in the last decades led to a dramatic increase in the number of patents dealing with recombinant allergens and peptides thereof.
3. The type of technology most frequently patented in the period ranging from 1998 to 2014 was the recombinant allergens and peptides thereof.
4. The European companies were the sector with the highest number of patents on recombinant allergens and peptides thereof.
5. Extract-based preparations are being replaced in allergen-specific immunotherapy by recombinant allergens, mainly as hypoallergenic derivatives.
6. The latest patents claiming engineered hypoallergens with low IgE-binding and conserved T-cell reactivity seem to be more convenient, efficient, and safer.

1. INTRODUCTION

Allergies, in particular atopic asthma, are chronic diseases caused by immediate hypersensitivity reactions to environmental antigens, which are harmless to non-allergic individuals (non-atopic). Allergic diseases are very common worldwide and their prevalence is increasing in industrialised as well as in low- and middle-income countries, especially in large cities of Latin America [1–3]. Until now, there is no curative treatment for these diseases, and the most effective palliative drugs are corticosteroids. This kind of pharmacotherapy has serious side effects when used for mid- or long-term treatments [4–6]. It is suggested that allergy occurs as a result of a deregulated type 2 immunity that involves type 2 cytotoxic T lymphocytes (Tc2) and T helper 2 (T_H2) cells, which produce interleukin-4 (IL-4), IL-5, IL-9 and IL-13. These type-2 cytokines, in turn, regulate immunoglobulin E (IgE) class switching by B cells, eosinophils and mucus production [7]. Recently, GATA-3+ type-2 innate lymphoid cells (ILC2s) were also considered to be involved in the development of allergies. It has been shown that ILC2s produce T_H2 cytokines in tissues and can be found on the skin and mucosal surfaces, including those of the respiratory tract [8,9]. Moreover, Th17-cells have been found to be important cells in allergen-induced airway responses [10,11].

Following allergen crosslinking of specific IgE, which is bound to its high-affinity receptor FcεR1 at the surface of effector cells, a variety of mediators are released, including histamine, leukotrienes, prostaglandins, proteases, and cytokines. These mediators induce rapid-onset marked symptoms of the immediate hypersensitivity (type 1) reaction [12].

An alternative therapeutic approach to current drug usage for allergic diseases is allergen-specific immunotherapy (SIT) [7,13]. The main goal of SIT is the induction of immune tolerance as a primary target for the prevention or treatment of these illnesses. In fact, since its first use, more than a century ago, SIT has been used to treat allergies by suppressing allergy-associated symptoms, during three to five years of treatment [7]. The effect of SIT is long-lasting and protects the individuals against the development of allergic symptoms for

seven to twelve years, but is not life-long [7,13–15]. The most commonly used approach for SIT is based on the use of crude extracts consisting of heterogeneous mixtures of allergens and non-allergens prepared from different sources [16,17]. One of the major drawbacks of this approach is the fact that the molecular composition of such crude extracts may vary considerably between the various sources and batches. Furthermore, the crude extracts must be administered for long periods to achieve effective results. Above this, they might cause severe side effects in patients, including anaphylactic shock that may lead to death [18,19]. In a study by Queirós and collaborators (2008), it was reported that several house dust mite extract preparations produced in Brazil either lacked proteins or had them in very low concentrations. Besides, the main antigen of *Dermatophagoides pteronyssinus* (Der p 1) was absent in most of the preparations evaluated [20]. Another study by Brunetto and collaborators (2010) showed that mite extracts from different manufacturers were heterogeneous in their protein composition, which strongly affected *in vivo* allergy diagnosis due to the absence of relevant allergens in the preparations [21].

The advent of molecular biology opened up new opportunities for SIT including the possibility to produce well standardised and effective allergen preparations through recombinant DNA technology [16,22]. There is now a variety of recombinant allergens in use for SIT that were produced from different sources such as pollen, cats, dogs and mites [13,23]. In this scenario, the combination of a panel of recombinant allergens for SIT has been used [24], with the aim of partially reconstituting allergen extracts. As an example, a vaccine has been produced with five recombinant allergens from *Phleum pratense* grass pollen, which showed effectiveness in reducing the symptoms and the need for symptomatic medication in grass pollen-allergic patients [25].

Nevertheless, SIT may also induce side effects, ranging from mild and local to severe and life-threatening symptoms, such as anaphylactic shock [26]. To overcome these undesirable effects and to increase effectiveness, modern protein engineering tools have been used in conjunction with novel bioinformatics tools with the goal to develop hypoallergenic allergen derivatives [26,27]. These hypoallergenised proteins can be engineered by trial and error

approaches, such as gene shuffling, through testing different peptides formulations from the respective recombinant antigens [26,28]. Otherwise, hypoallergens may be rationally designed by *in silico* mutagenesis, wherein in a first step an epitope mapping is realized. Following that, expression, purification, and biochemical and immunological characterisation of the selected molecules are achieved, using techniques such as mass spectrometry, circular dichroism and the dendritic cell-based degradome assay [27,29]. Hypoallergenic recombinant allergens have already been used in several clinical studies to reduce side effects of SIT, including the risks of anaphylactic shock, without reducing their effectiveness compared to natural or recombinant allergens. The advantages of the hypoallergenised recombinant allergens, in comparison with the non-hypoallergenised molecules, are their low IgE-binding and high T cell activation capacity. The combination of these properties has the potential to induce tolerance to the respective allergen [22,28,30,31] in the absence of side effects related to the allergen formulation. The latter has additional clinical implications, such as side effects, which is an important reason for SIT to be performed at clinics under the supervision of medical staff [32].

In this review, we aimed to map the technology landscape of the production of allergen preparations for allergy-specific immunotherapies. A patent review was realized, through the patent application documents deposited within the last years at the European patent office (EPO), to achieve this goal.

2. METHODS

Data retrieval and analysis

Data were obtained in the free access patents database of the European patent office (EPO) (<https://www.epo.org/searching/free/espacenet.html>) on April 2015. This database was chosen because it periodically receives data from more than ninety country patent offices worldwide, thus allowing for a comprehensive global search.

Our purpose was to evaluate, through the patent application analysis, recent advances in technologies related to allergen immunotherapy. The initial search strategy used was

'classification search' to cover all patents focused on the technological fields related to allergen immunotherapy. The Cooperative Patent Classification (CPC) code chosen was A61K39/35 (allergen).

The EPO advanced search option was used to combine this code with the patent application's publication date to filter the comprehending documents published since 2010. There are some limitations in this website; for instance, lists can only be exported page by page. Therefore, users can only export up to 30 records at a time in the compact mode, and the fields available for export are limited. However, we evaluated that this level of export power was sufficient to support our objectives.

The compact mode was activated to download the largest number of patent applications. Data were then exported to a Microsoft Office Excel 2013® spreadsheet. Figure 1 outlines the search strategy used in this study.

The CPC system has a hierarchical structure consisting of sections, classes, subclasses, groups and subgroups. This system is used by the EPO and partner patent organisations for carrying out patent application searches. Moreover, CPC is based on the International Patent Classification (IPC). The scheme was initially based on the former European Classification System (ECLA), which was an extension of the IPC and kept all of its properties (hierarchy, titles, and expandability). Our strategy of using a classification search was aimed at increasing the number of patent applications analysed, since not all applications are written in English. In addition, other databases such as Patent Scope and Google Patents do not have this kind of search option, even though Patent Scope has the same codification based on IPC. The CPC codes found are described in Figure 1.

Data duplications were removed using the 'remove duplicates' tool in the Microsoft Office Excel 2013® software. As the patent applications may have been done in several countries, leading to different patent application dates, in this study we considered the date of the first application. The data were initially analysed to sort different types of technologies used in allergen immunotherapy by reading the abstract and claims of each patent, and when there were doubts, the full patent text was analysed. Table 1 elucidates this initial classification. In

this first round of classification, other sub-classifications have been made to determine the type of allergy that the patents were focused on. Then, information on the allergens mentioned in these patents were retrieved.

The second analysis focused on the applicant's nature, i.e., patent applicants from academia, companies or independent inventors. Only the first type of applicant was considered in the descriptive analysis.

During these two classifications, it was found that, although some patents are placed within the CPC code A61K39/35, they did not refer specifically to allergen immunotherapy. In this case, they were excluded from the dataset. Out of the 608 results obtained using our search strategy, only 375 patent applications fulfilled our criteria. This search protocol and this analysis approach have been used by former publications in the area of intellectual property [33,34].

We found 144 duplicate patent applications, which were excluded from the dataset. Finally, during the classification by type of technology in allergen immunotherapy (Table 1), 30 patents could not be used in this field of invention and, therefore, were excluded from the descriptive analysis. Although these patent applications were related to allergy, their claims involved methods for allergen extraction and production of monoclonal antibodies for the development of diagnostic kits but not for the use in allergen immunotherapy. In total, 201 patent applications were analysed in the present study. The final screened data were exported for visualisation using the Gephi network mapping software (<http://gephi.github.io/>) and the Microsoft Office Excel 2013®'s graphs. Gephi visualisations deployed the Fruchterman-Reingold algorithm with nodes (patent applications) and edges (interactions), which were manually adjusted to ensure label clarity.

3. RESULTS AND DISCUSSION

3.1. Patent applications: geographic distribution and analysis by technology

Table 2 describes the number of patent applications retrieved by our search strategy according to the specific CPC codes and distributed throughout the years analysed in this study.

The descriptive analysis of the patent application for allergen immunotherapy showed an increase in deposits, starting in 2001. These data are evidenced by the cumulative numbers of patent applications by priority date, as shown in Figure 2A, reaching in ten years with a volume of 35 applications. Additionally, the aspect of the curve shows that the technologies related to allergen immunotherapy are still emerging, which configures an area with potential for growth and market value. This increase can also be associated with the advancement of recombinant DNA technology and its widespread use worldwide. Moreover, the broad use of this technology may have led to an increase in the number of patents in allergen immunotherapy, especially for recombinant-based allergen-specific immunotherapy [35].

The total of 201 retrieved patent applications was then placed in a co-occurrence matrix, which provides a quantitative measure of the number of applications by country. The United States of America (USA) showed the highest number of patent applications in the field of allergen immunotherapy during the period evaluated, with 55 deposits identified for this country (Figure 2B). As this analysis was performed using the publication number, it was observed that several applicants have chosen to apply for an international patent application. It was found 20 patent applications for the World Intellectual Property Organisation (WIPO), 14 patent applications for the European Patent Office (EPO), and 2 patent applications for Eurasian Patent Office (EAPO). There are some advantages of choosing these international institutions as a patent recipient compared to direct deposit at the corresponding national offices. The benefits are the following: (i) a single act of filing an international application form under the Patent Cooperation Treaty (PCT), and (ii) the possibility of reducing the number of filings of separate applications in countries or regions in which the applicant requires protection. Hence, a single patent application can be valid in several countries in a single publication. In addition, all designated offices must accept this international patent form for the purposes of the national phase; thus, there is no need to fulfil different formal

requirements of patent applications in the many countries where protection may be necessary. Moreover, it is possible to reduce the costs of patents by having only one international application.

Other countries with significant patent application numbers were Canada, with 23 deposits, Japan with 16 deposits, and Australia with 13 deposits. The higher patent production are found mostly in developed countries where the consumer market may be an important factor to explain the higher number of deposits, since the prevalence of allergy in those countries is high, especially allergy caused by food allergens [36–40]. Additionally, such countries might have intellectual property offices with reduced bureaucracy, which may have adopted their own intellectual property laws, and developed accelerated review processes of patent applications. The Canadian Intellectual Property Office (CIPO), for example, has currently revisited administrative procedures for patent applications, which had examination periods lasting for 2-3 years. Now, it has become possible for an applicant in certain cases to accelerate the examination process of an application and to obtain a first office action in as little as 2 to 3 months [41]. Interestingly, according to data retrieved from Espacenet®, none of the patent applications examined in this review included companies, research institutions or inventors from Canada. Concerning Japan, Nicholas (2011) reviewed the reasons for the Japanese technological modernisation and showed quantitative metrics derived from patent data in his paper. His analysis showed that the Japanese economic development by patented technologies was a result of both a focus on international technology transfer as well as on investments in domestic technological capabilities [42]. Furthermore, in October 2004, the Japan patent office (JPO) established the Advanced Industrial Property Network (AIPN) with the purpose to provide a machine-based translation system, enabling Japanese applicants to more quickly obtain patents in other countries [43].

Regarding the nature of the patent applicants, we observed that companies hold 64% of the total number of patent applications, followed by independent inventors with 19% and finally academia with a 17% share (Figure 3A). The patent applicant analysis concerning the companies showed that the largest technologic domain related to allergen immunotherapy

belongs to European companies (Figure 3B). The Danish company ALK ABELLO AS, a pharmaceutical company that has also been dedicated to research focusing on the prevention, diagnosis and treatment of allergies since 1923, holds the largest number of patents ($n=19$). During the years analysed in this work, this company applied at least one patent per year since 2005, which shows a strong policy focused on appropriation of the technological production developed in this area. Furthermore, since 2006, this company has had a tablet-based sublingual allergy vaccine, which is part of a SIT program [44]. However, in our data, ALK ABELLO AS filed only 4 (out of 19) patents related to recombinant peptides, whereas all the other patents were classified as extract-based products. Despite being in second place, CIRCASSIA LTD stands out as a promising company in the allergen immunotherapy field; considering that, since its creation in 2006 in the United Kingdom, it has already applied for 15 patents in the area. CIRCASSIA LTD has four SIT programs in their pipeline products, in which the SIT to treat cat allergy is in phase III (ToleroMune product) [45]. Moreover, different to ALK ABELLO AS, 14 patents filled by CIRCASSIA LTD were related to recombinant peptides and one of them is a hypoallergen (to treat birch allergy), which is the most innovative approach in this area. In this matter, the Austrian company BIOMAY AG has applied seven patents, of which five were related to hypoallergens. One of these products has had a clinical safety evaluation [46]. Moreover, this company has developed the BM4 hypoallergen for birch pollen SIT. The patent application describing the BM4 hypoallergen provides an example of how scientific research can be converted into patents, as all of the inventors were researchers from the University of Salzburg and the first applicant (assignee and current owner) was the company BIOMAY AG. Of note, the above BM4 patent with patent family number WO2008EP05324 and priority number EP20070450145 did not appear in our extracted data from ESPACENET [47].

In our data, other companies have shown deposits with hypoallergens. Among them are: PHADIA AB, MERCK PATENT GMBH, and ANERGIS SA with 2 patent applications each and BIOMEJ AG, BIAL IND FARMACEUTICA SA, and TEKNOLOGIAN TUTKIMUSKESKUS VTT with one deposit each. However, only the companies MERCK PATENT GMBH and

ANERGIS SA are presently conducting clinical trials for their allergy immunotherapeutic products [45,48]. Additionally, among the other 41 companies found in our data with patent applications related to allergen immunotherapy, it should be mentioned that STALLERGENES SA and ALLERGY THERAPEUTICS UK LTD have novel SIT products that are in late-stage development [45].

It is also noteworthy that only a few USA companies figure among the main patent applicants (Figure 3B). DYNAVAX TECH CORP has more than two applications and other USA companies have two or less. This probably occurs because this country presents a greater number of patents applied by independent inventors and academia, rather than by companies.

It was observed that the academic applications were not concentrated in a particular university or research institution; nevertheless, they were more concentrated in the United States of America (41%). Despite the performance of USA research institutions, it was noticeable that the global landscape is not positive for academia, mainly for Latin American institutions, where the total number of patent applications was rather unimpressive. Furthermore, Latin American patent applications (shown in Figure 2B) were not actually deposited by native applicants, but by foreign companies and/or universities. As mentioned previously, it is possible that these applicants have applied for separate patents, to seek additional protection beyond their respective countries.

Among the patent applications from independent inventors, only one was from Latin America, which claims recombinant DNA molecules codifying fused peptides from major group 1 and 2 allergens from *Blomia tropicalis* and *Dermatophagoides pteronyssinus*, but the applicants had chosen an international application instead of an application through national office since none of the application numbers had the origin country code of the inventors [49]. Therefore, incentives and reinforcement of the university internal policies by governmental and private funding agencies are needed to improve the present situation in Latin America in relation to intellectual property, especially to leverage the allergen immunotherapy area [50,51]. Of note, several research developments were published as peer-reviewed articles but were not

appropriately converted to patents [50,51]. According to Germann and collaborators (2013), there are two key factors needed for successful collaborations between the publicly funded research in academia and the development of applied activities within the pharmaceutical industry: (i) the preparation of both partnering organisations to accommodate novel ideas; and (ii) the creation of trust-based relationships between the institutions and the companies [52]. Based on these premises, it would be possible to increase the number of patent applications by universities and research institutions, even in partnership with pharmaceutical companies.

Considering the types of technologies used in allergen immunotherapy (Table 1), a large number of patent applications claiming recombinant peptide(s) were observed, followed by total extracts, formulations, devices, and hypoallergenic molecules (Figure 4A). These results clearly show a tendency in the allergen immunotherapy area, since the first patents for allergen immunotherapy were covering total extract allergens, which have recently been replaced by the recombinant peptides technology.

The changing of technology type employed for allergen immunotherapy can be explained by the fact that extract-based allergen immunotherapy is associated with several problems: (i) the molecular composition of these products may vary considerably, since they are very heterogeneous protein mixtures; (ii) it is extremely difficult for allergen manufacturers to define the exact potency of their extracts, given that these extracts are not standardised and the amount and proportion of major allergens may vary among different preparations; and (iii) both total IgE binding to the extract or IgE binding to the major allergen cannot be quantified satisfactorily [16]. In contrast, the recombinant peptide technology offers several advantages, such as: (i) the molecules can be fully characterised and produced with a high batch-to-batch consistency; (ii) the reagents can be easily quantified, allowing standardisation of the therapeutic product; (iii) the recombinant proteins can be formulated according to the need of the patients; and (iv) recombinant DNA technology enables the production of low IgE-binding allergens with adequate T cell activation properties, thereby leading to an improved safety profile and the efficacy of allergen immunotherapy [13,16,53].

Within the patent group classified as recombinant peptide(s), we also evaluated the type of allergic diseases the patent claims were intended to treat. As shown in Figure 4B, 62% of the recombinant peptide patents were aimed only at treating allergies caused by environmental allergens and only 11% of patents have specific claims to treat food allergy with recombinant peptides and 27% have claims for the treatment of both types of allergies (for environmental and food allergen). For example, the patent with the patent family number WO2008GB02780 and priority number GB20070015949 (from CIRCASSIA LTD company) claims sequences of recombinant peptides from mites, mould, cockroaches, and also from peanut allergens [54]. A closer look at some applications showed a tendency to include a multitude of allergen sources, thus increasing the market coverage of the products. This trend is in line with reports describing the increasing prevalence of allergies caused by environmental allergens [55,56] and food allergens, such as peanuts [57,58].

Our analyses revealed that the highest number of patent applications was related to the treatment of allergies caused by environmental allergens. These findings can be considered positive for the advancement of allergen immunotherapy, not only because of the high prevalence of such allergies worldwide [3,59–61], but also due to the fact that allergies caused by environmental allergens are often accompanied by associated food reactions. Although clinical data is still missing, it is conceivable that the developed immunotherapies could also ameliorate food allergies that are caused by cross-reactivity with environmental allergens. Examples are cases of cross-reactivity between fruit/vegetable allergens and pollen [62,63], as well as house dust mites and shrimp [64,65].

We also conducted an analysis to evaluate which sources of environmental allergens appeared more frequently in patent applications claiming recombinant peptides for immunotherapy. As shown in Figure 4C, the environmental allergens having the largest number of patent applications with recombinant peptides are those derived from mites (41%), pollen (20%), cats and fungi (10% each), and bee and horse (3% each). Furthermore, 13% of the patent claims included more than one allergen source. As expected, mites and pollen allergens accounted for the majority of patent deposits, probably because they are the

source of aeroallergens causing most of the allergic manifestations worldwide (mites) and in developed countries (pollens) [55,60,66,67], thus reflecting the size of the market for therapeutic formulations containing such peptides.

3.2. Recombinant hypoallergenic molecules

As shown in Figure 4A, hypoallergenic variants of recombinant allergens already occupy the fourth position in the total number of patent applications. The patent applications using this technology started to be deposited only in 2010, according to data retrieved from Espacenet®. Unlike recombinant peptides derived from native allergens, in which mite-derived peptides display the largest number of patent applications, for hypoallergenic variants; the largest number of patent applications was observed for pollen allergens (Figure 5). Additionally, the patent application with patent family number WO2007AT00281 and priority number AT20060000994 was the only one in our analysis that claimed the use of more than one hypoallergenic variant from the group consisting of the major birch pollen allergens, the major house dust mite allergens, and the major cat allergen, among others [68]. These pollen allergen patents might reflect the advanced state of research on recombinant hypoallergenic technologies in Europe [19,23,69] and the probable transfer of technology from universities and research institutions to companies. After all, the prevalence of allergies caused by pollen is very high in this continent [3,67]. These findings also are in agreement with the results found for the number of companies, where European companies clearly dominated the patent field of allergen immunotherapy (Figure 3B). Moreover, it was also observed that one-third of the patent applications by European companies had universities as co-applicants. Such university-industry partnerships aim to advance the efforts to improve public health, which is also one of the goals of research conducted by universities.

Several approaches have been described to produce hypoallergenic molecules. Presently, it is well established that such hypoallergenic molecules display interesting features, such as:

- (i) strongly reduced allergenic activity allowing the administration of higher doses than the

natural allergen; (ii) induction of similar T cell proliferation to the native allergens but reducing significantly the levels of the T_H2 cytokine profile; (iii) reduced allergen-induced boosts of IgE production and IgE-facilitated allergen presentation to T cells; (iv) increased production of allergen-specific IgG antibodies, in particular IgG4, which is supposed to block the IgE-binding to allergens [19,70,71]; and (v) reduced capacity to induce effector cell degranulation [28,30,31,72,73]. All, or at least most of these parameters have been fulfilled by patents related to hypoallergenic molecules. In this review, we will discuss some approaches and examples of engineered hypoallergens that appeared in the patent claims of our exported data from Espacenet®.

A patent filed in 2012 claimed a hypoallergenic derivate that aimed to treat *Dermatophagoides pteronyssinus* allergy (family number WO2011EP71377 and priority number EP20100193292) [74]. This derivative was produced by fusing proteins and some modifications using two techniques: DNA shuffling and site-directed mutagenesis. In the DNA shuffling technique, it is possible to mimic natural evolution, and there is no necessity of knowledge about structural or functional characteristics of the target molecules. The method produces a library of novel chimeric genes, from which variants with the desired properties can be selected by screening methods [75]. In the case of site-directed mutagenesis, it is possible to make specific and intentional changes in the DNA sequence of a gene. The mutation shall be a single base change, multiple base changes, deletion, insertion, or even the construction of a synthetic primer. The primer contains the desired mutation, which can hybridise with the DNA of the gene of interest [76].

Using these two approaches, the inventors have generated a chimera containing T cell epitopes of two major allergens from *Dermatophagoides pteronyssinus*. In addition, the removal of all cysteine by site-directed mutagenesis facilitated the large-scale production in clinical trials by preventing the formation of aggregates through disulphide bonds, allowing the hypoallergenic derivative to remain monomeric in solution. A major advantage of the DNA shuffling approach is the possibility to select molecules with reduced anaphylactic potential and improved immunogenicity, thus producing optimal molecules for more efficient

and safer forms of allergens vaccines [77]. However, there also disadvantages such as: (i) the conditions to produce hypoallergenic molecules are neither predictable nor controllable; (ii) the necessity of performing time-consuming blinded trials to finally obtain suitable candidate molecules [27].

Another way to obtain hypoallergenic molecules is using the allergen fragmentation approach. This technique has been utilised in some patent applications, such as those with the patent family numbers WO2005AT00486 [78], WO1998SE01765 [79] and WO2004EP04848 [80]. The intent of this technique is to reduce the anaphylactic potential of allergens through the gene fragmentation allowing the disruption of the allergen's three-dimensional structure [81]. However, this approach is not knowledge-based and the fragmentation sites or fusion partner molecules are selected by arbitrary decisions (trial and error method). Although the Bet t v1 fragments have exhibited random coil conformation and almost no allergenicity in the WO1998SE01765 patent (priority number SE19970003531), they displayed different structures than the entire wild-type molecule [79]. Furthermore, in the WO2004EP04848 patent (priority number DE2003125508) it was possible to note that the fragmentation approach cannot be generalised because various fragmentations of Phl p 5 did not generate any hypoallergenic derivative at all [80].

In the WO2003EP14507 patent (priority number EP20030001242), the applicants claimed a process for the preparation of a hypoallergenic mosaic antigen derived from an allergen, as well as hypoallergenic variants from *Phleum pratense* [82]. In the first step, the allergen was fragmented into at least two parts, and the IgE reactivity of each part was determined. In the second step, those parts of the allergen, which had no detectable IgE reaction, were recombined (shuffling) to a mosaic antigen, but the order of the amino acids of the mosaic antigen was different from that of the natural allergen. This mosaic antigen had the advantage that a sufficient amount of blocking IgE antibodies was produced [83]. However, it is still difficult to prepare several fragments for one allergen, and the random shuffling of allergens can be quite time-consuming [14]. Moreover, there is no guarantee that the

derivative that did not keep the structure of the wild-type allergen would be able to induce tolerance properly. Therefore, this technique may not be worth generalising.

Unlike these approaches, which are based on trial and error, there is a WO2008AT00049 patent application (priority number AT20070000231) which claims a method for identifying a hypoallergenic derivative of a wild-type allergen based on prior knowledge of the mutation [84]. Basically, in this approach it is necessary to determine the three-dimensional structure of the wild-type allergen. After that, at least one point mutation is introduced into the wild-type allergen, thereby obtaining a mutated allergen. Finally, when destabilisation of the three-dimensional structure of the mutated allergen is found, a hypoallergenic derivative of the wild-type allergen is then identified. It is also important to note that the change of the three-dimensional structure of the mutated allergen is compared to the wild-type allergen by determining energy differences between the wild-type allergen and the mutant allergen expressed as a Z-score [27]. A major benefit of *in silico* mutagenesis is that the mutation and selection criteria can be easily adopted, even updated with any knowledge and new data about structural features. Another benefit is the rapid adoption of selection criteria, such as preservation of relevant T-cell epitope sequences and destruction of IgE-binding sites, and in some cases both [27,85]. Based on these clearly rational and measurable parameters, this approach represents a reliable method for screening hypoallergenic vaccine candidates with defined molecular properties, allowing the improvement of allergen-specific immunotherapy. The knowledge of the three-dimensional structure of the molecule is very important and a challenge because it depends on the number of available structures in the data bank. However, the number of available structures of allergens in the databanks is rapidly growing. The key patents cited in this section are in accordance with the published research trends and novel developments in SIT [7,13,16,19,22,23,53]. In fact, Ferreira et al. [19] have provided a comprehensive overview of clinical trials using recombinant allergens, detailing the type of clinical study and the engineered molecules employed as hypoallergenic vaccines.

Clearly, this is a very moving time for the patent field of allergen immunotherapy. Many novel and likely better approaches aiming to produce hypoallergenic derivatives are under development. One of these approaches consists of using hypoallergenic derivatives in the sublingual immunotherapy (SLIT), which has been progressively introduced in allergen-specific immunotherapy during the 2 last decades [15]. SLIT claims to have increased safety and efficacy, features that have been confirmed by clinical trials and meta-analyses studies [86–88]. Major advantages of this delivery route for patients (especially for children) is the avoidance of injections [89] and the possibility of administration of the vaccine outside medical settings [90].

Accordingly, in our study, the claims of the patents classified as hypoallergens included the sublingual route of application. Some patents claimed only the sublingual route, while others claimed subcutaneous, sublingual, oral, nasal, rectal, topical, inhalation, or parenteral administration. Moreover, some applications did not include claims on administration routes because the patents focused on processes of production, such as the hypoallergen formulations.

4. SUMMARY AND CONCLUSIONS

It was noticed that there is no specific code for the patent field of allergen immunotherapy. Although there is the A61K39/36 code (for pollen allergen) linked to A61K39/35 code (for allergens in general), there is no specific code for mite allergens. This is an important point since the allergy caused by mites has a high prevalence worldwide [55,60,91] and patents in this area have already been granted [92–94]. Also, as life expectancy increases and as more and more synthetic products are being used by the healthcare system, the industry of immunotherapeutic products is significantly growing. Therefore, institutions responsible for registering intellectual property rights, such as the WIPO, should evaluate the needs for sub-classifications, referring to house dust mites and other allergens, as well as for anti-allergy immunotherapies in general.

In recent decades, patent applications of technologies that can be used in allergen immunotherapy have increased significantly. This can be associated with advances in recombinant DNA technology and protein engineering methodologies, including directed evolution using DNA shuffling and site-directed mutagenesis. The number of patents identified in the studied period (1998 to 2014) utilising the technology of recombinant peptides may be reflected by the use of these techniques. European companies were seen to be the sector that produced the highest number of patents involving recombinant peptides, and one-third of these applications had universities as co-applicants. Furthermore, the advent of hypoallergenic derivatives of environmental allergens, which tend to be safer than natural and native ones, has significantly contributed to the increase in the number of patents in the last few years. It seems clear that the use of crude allergen extracts is gradually being replaced in allergen-specific immunotherapy, at least in patent applications. Therefore, it is possible to hope the advance of safer technologies that are more effective and targeted to the patient's individual profile of allergic sensitisation.

5. EXPERT OPINION

Allergen-specific immunotherapy represents the only potentially curative therapeutic intervention. By reviewing and analysing the patent applications in recent decades, it became clear that we can anticipate a positive development for the improvement of allergen-specific immunotherapy vaccines. It can be expected that SIT will be a more convenient, efficient, and safer form of treatment for allergies. Our analysis showed that the extract-based immunotherapy is being replaced by the use of recombinant allergens, and the development of their hypoallergenic forms was an important upgrade in the allergy immunotherapy. This change is becoming possible for following reasons: (i) new approaches for the rational design of vaccine candidates for allergen-specific immunotherapy; (ii) more detailed study of the allergic immune response; and (iii) the development of novel concepts for allergy diagnosis in the recent decades. Furthermore, the unremitting advances of recombinant DNA

technology will help to overcome the challenges of the long-term protocols in allergen-specific immunotherapy, allowing better patient adherence. It is clear that the design of low IgE-binding hypoallergens will profoundly expand the scope of allergen-specific immunotherapy and possibly also break the ground towards effective allergy vaccination. In fact, there are some engineered hypoallergens that have already been shown to be clinically effective in allergen-specific immunotherapy. It is suggested that hypoallergens will allow a reduction in the number of doses applied in allergen-specific immunotherapy protocols, reducing the duration of the treatment. Further developments in the field of allergen-specific immunotherapy could combine engineered hypoallergens with novel adjuvant formulations. Furthermore, these recombinant proteins should be obtained using alternative systems for expression, which should be as close as possible to the native organism. These alternative methods will be a disruptive technology in recombinant vaccine development because recombinant proteins cannot always replicate their natural functions and, in some cases, candidate vaccines have not shown suitable protection [95,96]. Post-translational modifications (such as glycosylation, methylation, acetylation, phosphorylation, lipidation, formation of disulphide bonds and elevation of peptide bonds) do not occur in recombinant proteins which are produced in prokaryote expression systems, and are important to induce immune responses associated with protection or tolerance [97–99]. Indeed, the expression of some proteins with complex posttranslational modifications does not take place in *E. coli* expression systems [100]. Therefore, the ideal approach will be to produce the engineered hypoallergens in systems for expression that are closer to the organism which are the original source of the allergen. As an example of alternative system for the vaccine production, a *Haemonchus contortus* vaccine candidate was produced in the nematode *Caenorhabditis elegans* [101].

Lastly, an important point in the field of allergen-specific immunotherapy is the route of application. Probably, delivery systems such as liposomes and emulsions will become technologies with future potential. In our study, only five patents have claimed the use of liposomes as a delivery system for allergy treatment, showing the potential of this technology

in allergen-specific immunotherapy. The advantages of the liposome as a delivery system has been showed not only for drugs and genes [102,103], but also for pulmonary delivery system and has some advantages: (i) it can be prepared from compounds endogenous to the lungs; (ii) evasion from complement inactivation after *in vivo* administration; (iii) it has low cost; and (iv) relative facility to produce liposome complexes in large scale [104,105]. Therefore, the engineered hypoallergens could be delivered by the aerosol delivery of liposomes in the liquid state.

The advances in the production of engineered allergens, herein discussed, in allergy treatment, including increased long-term efficacy and safety, might be expected soon, especially if associated with technologies with future potential.

Declaration of interest

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* Of interest

** Of considerable interest

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Table 1. Patents related to allergen immunotherapy stratified by type of technology

CLASSIFICATION	DESCRIPTION
Recombinant peptides	Patents whose claims refer to one or more peptides, produced by molecular biology or genetic engineering techniques, for the treatment of allergies caused by environmental or food allergens.
Extracts	Patents whose claims include total protein extracts of one or more organisms for the treatment of allergies caused by environmental or food allergens.
Devices or formulations	Patents dealing with non-protein formulations for the treatment of allergies or devices that can be used for any allergen immunotherapy protocols.
Recombinant hypoallergens	Patents whose claims refer to a recombinant peptide with low IgE-binding to allergens and preservation of T cell epitopes
Adjuvants	Patents whose claims include molecules which promote the development of tolerance in allergen immunotherapy protocols.
Cells	Patents related to mammalian cell lines or probiotics which that carriers of DNA sequences encoding peptides for the treatment of allergies.
Nucleic acids	Patents claiming only sequences of siRNA, DNA and immunostimulatory sequences (ISS) that can be used in allergen immunotherapy protocols.
Carriers	Patents whose claims refer to liposomes, viral particles, viruses, and emulsions, which can be conjugated thereto: purified proteins, recombinant proteins, or any molecule for

the treatment of allergies.

Isolated peptides

Patents claiming formulations that contain purified native proteins, which can be used for the treatment of allergies.

Table 2. Scope of technological prospecting and CPC codes for patents related to allergen immunotherapy									
CPC codes/year	Chosen CPC codes				More specific CPC code combined by year				
A61K	X								
A61K38/00		X							
A61K39/00			X						
A61K39/35				X	X	X	X	X	X
2010					X				
2011						X			
2012							X		
2013								X	
2014									X
NUMBER OF VISUALIZED PATENT APPLICATIONS ON ESPACENET	> 100,000	> 100,000	> 100,000	3,247	102	112	95	173	126
NUMBER OF EXPORTED PATENT APPLICATIONS					65	73	66	86	85
TOTAL OF EXPORTED PATENT APPLICATIONS									375
A – Human necessities; A61 – Medical or veterinary science; hygiene; A61K – Preparations for medical, dental, or toilet purposes; A61K38/00 – Medicinal preparations containing peptides A61K39/00 – Medicinal preparations containing antigens or antibodies;									

A61K39/35 – Allergens.

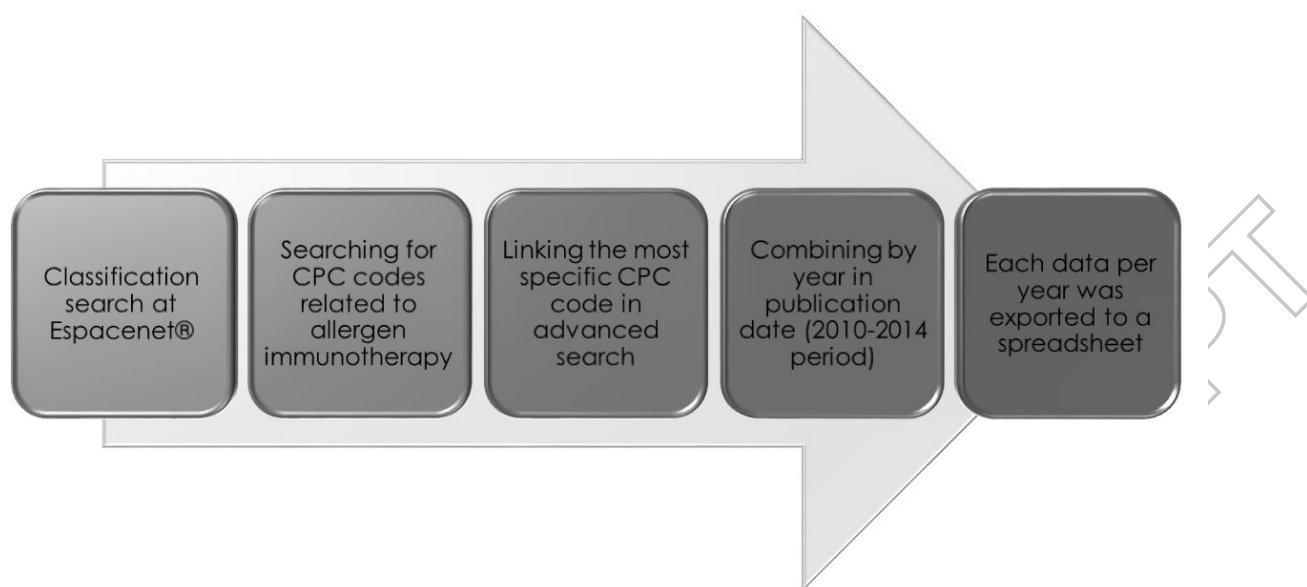


Figure 1. Search strategy using “Classification Search” at Espacenet®. The search was conducted in April 2015. The CPC codes were found as follows: A – Human necessities; A61 – Medical or veterinary science; hygiene; A61K – Preparations for medical, dental, or toilet purposes; A61K38/00 – Medicinal preparations containing peptides; A61K39/00 – Medicinal preparations containing antigens or antibodies; A61K39/35 – Allergens.

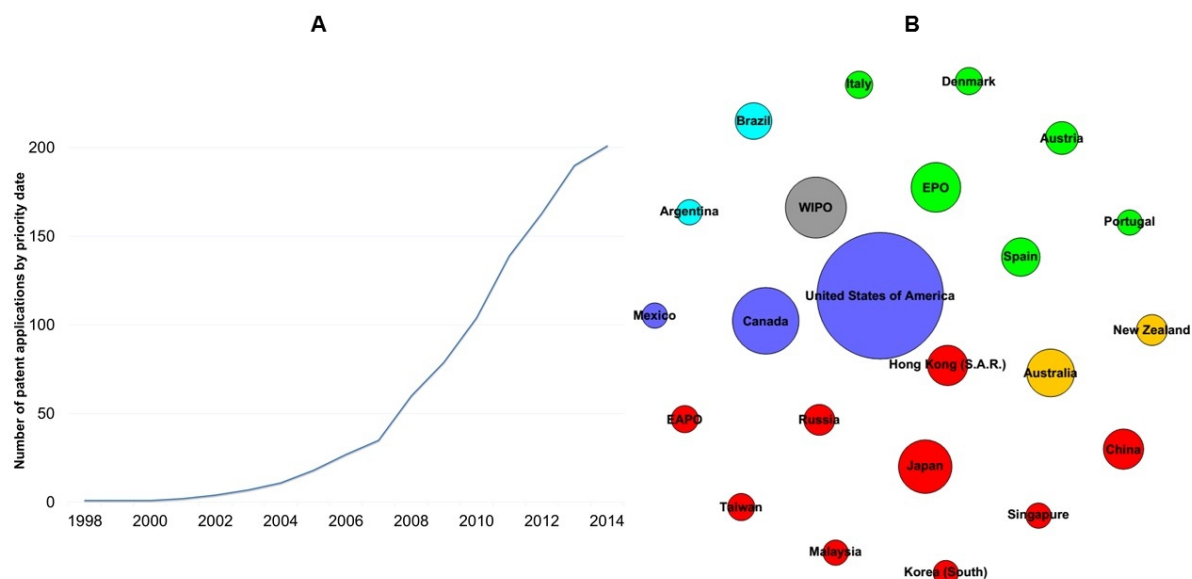


Figure 2. Cumulative yearly evolution and geographical distribution of patent applications in allergen immunotherapy. **A:** Cumulative evolution of patent applications related to allergen immunotherapy between the years of 1998 to 2014 (priority date). **B:** A Fruchterman-Reingold representation in Gephi of the top patent applications by country in ESPACENET®. The analysis was performed using the publication number. Node size represents the number of patent applications. Node positions have been adjusted to clarify labels. Node colour is based on the continental region as following: green for Europe, red for Asia, blue for North America, yellow for Oceania, arctic blue for South America, and grey for patents directly deposited on WIPO. WIPO: World Intellectual Property Organisation. EPO: European Patent Office. EAPO: Eurasian Patent Organisation.

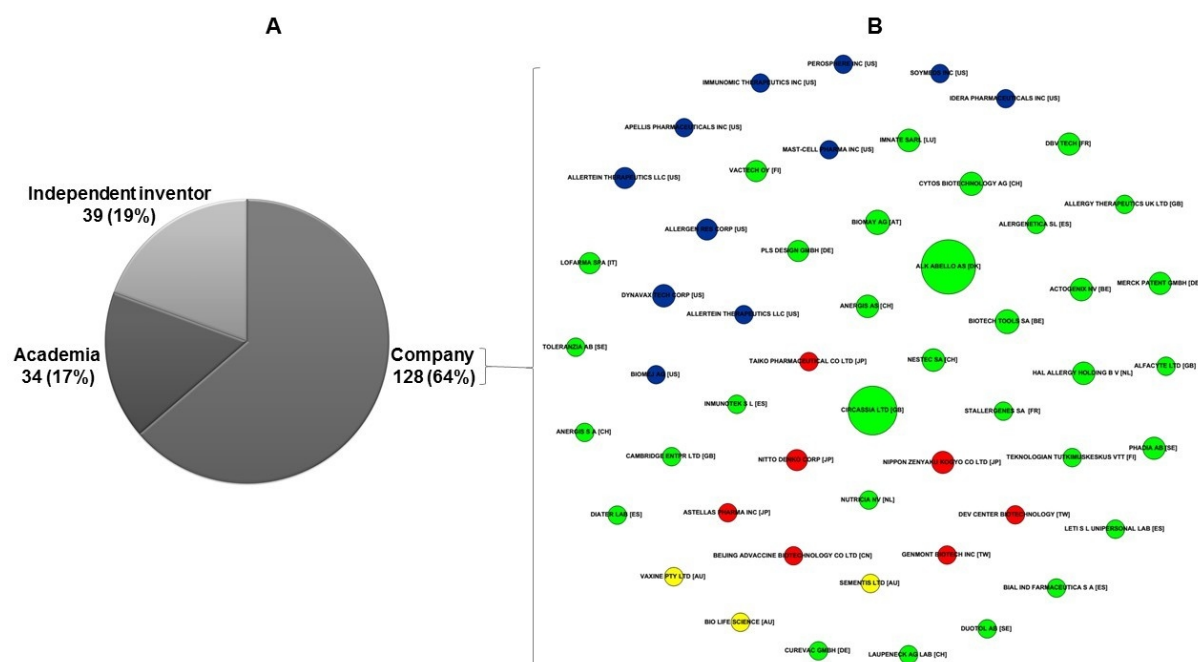


Figure 3. Worldwide distribution of patents by type of applicant and top companies in patent applications related to allergen immunotherapy. A: Academia is meant to be both universities and research institutions. Only the principal applicant was considered in this analysis. **B:** This is a Fruchterman-Reingold algorithm representation in Gephi. Note that only patents classified as company patents were considered in this analysis (N=128). Nodes were manually changed to prevent label overlaps and its size represents the number of patent applications. Node colour is based on the continental region as following: green for Europe, red for Asia, dark blue for North America, and yellow for Oceania. US: United States of America. LU: Luxembourg. FR: France. CH: Switzerland. AT: Austria. FI: Finland. DE: Germany. ES: Spain. GB: United Kingdom. DK: Denmark. BE: Belgium. NL: Netherlands. IT: Italy. SE: Sweden. JP: Japan. CN: China. TW: Taiwan. AU: Australia.

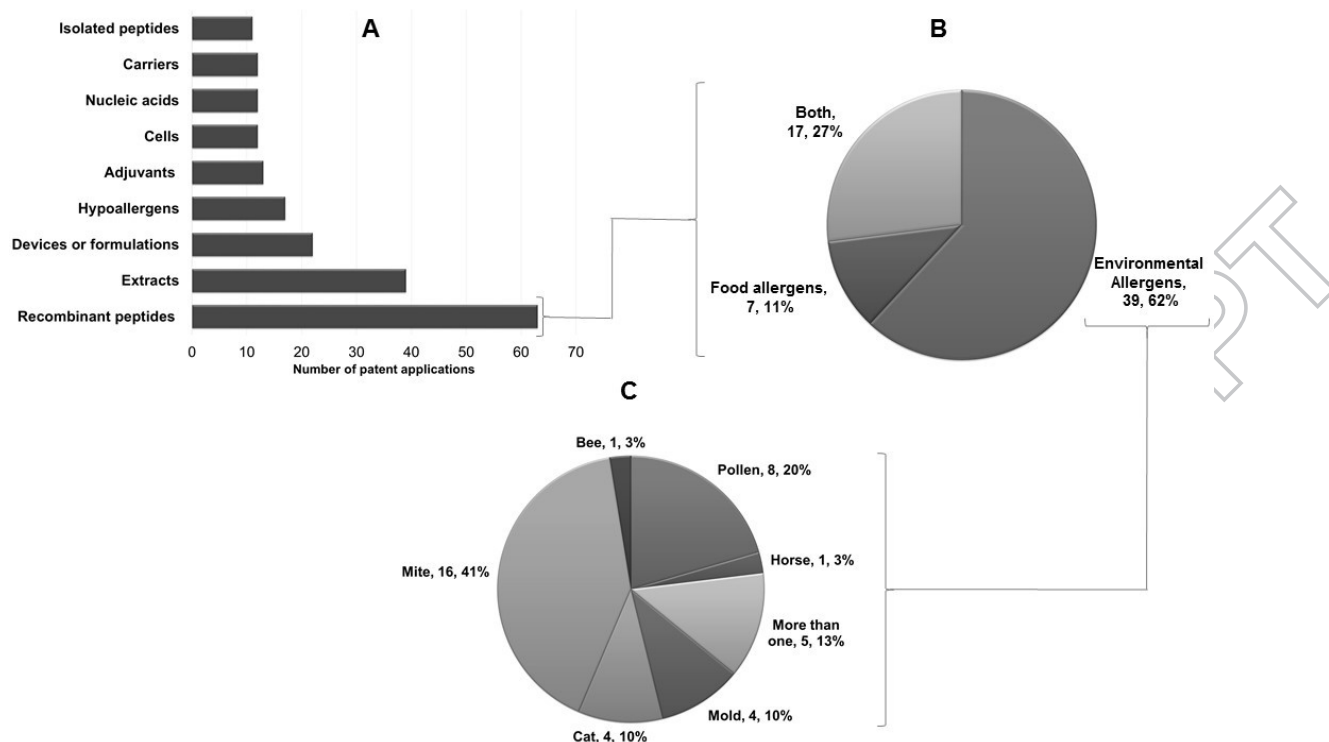


Figure 4. Worldwide distribution of patents related to allergen immunotherapy for the treatment of allergy caused by food or environmental allergens. A: Number of patents by type of technology (please see how the classification was made in Table 1). **B:** Distribution of patents by type of allergy (caused by food allergens or environmental allergens) that the recombinant peptide claims to treat. **C:** Distribution of patents with recombinant peptides stratified by type of environmental allergen.

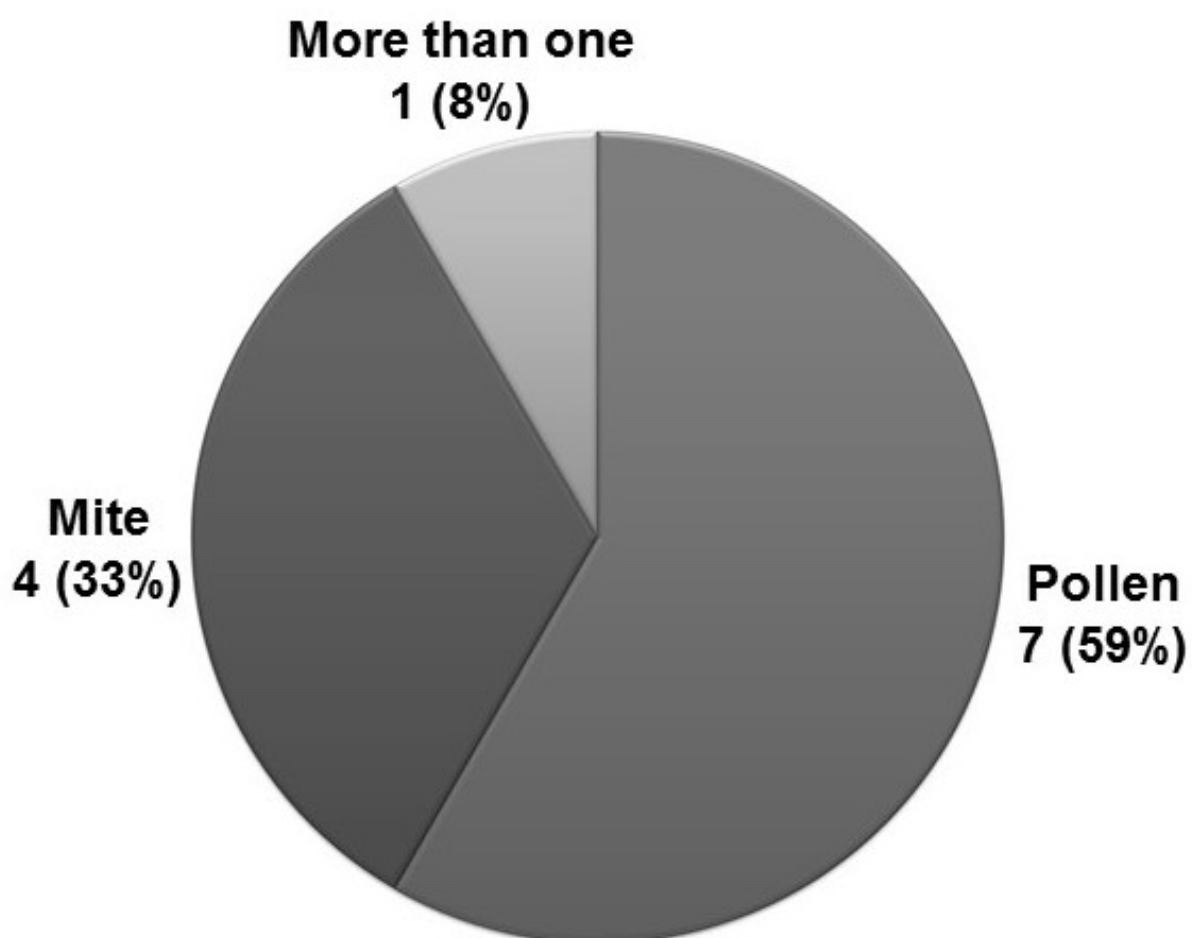


Figure 5. Distribution of patents that claim hypoallergenic proteins to treat allergies caused by environmental allergens. Note that only patents classified as environmental hypoallergens were considered in this analysis (N=12, 71% of deposited hypoallergens).