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Faculdade de Farmácia

IMMUNOGENIC RESPONSES IN THE CONTEXT OF BIOLOGICAL MEDICINES REGULATORY ASSESSMENT

Ana Carlota Coelho Correia

Dissertation supervised by Professor João Gonçalves

Regulation and Evaluation of Medicines and Health Products

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Resumo

As atividades regulamentares são cruciais para a avaliação da eficácia e segurança dos medicamentos biológicos, uma vez que, devido à sua origem e modo de ação, a ocorrência de reações imunogénicas é mais propícia aquando da utilização deste tipo de medicamentos. Assim, de forma a garantir a segurança dos doentes, é extremamente importante desenvolver estudos imunogénicos adequados. A *Food and Drug Administration* e a Agência Europeia do Medicamento desenvolveram *guidelines* que visam auxiliar os titulares na realização de estudos de imunogenicidade, sendo em geral, a metodologia utilizada por ambas as agências semelhante. O tipo de proteína terapêutica e o método de administração podem influenciar o aparecimento de anticorpos anti-medicamento (ADAs) e/ou anticorpos neutralizantes (nAbs) responsáveis pela ocorrência de reações imunogénicas.

Para os medicamentos biológicos com atividade direcionada (grupo 1), foram detetados níveis mais elevados de ADAs $(\mathrm{U}(45) = 154.000; \mathrm{p} < 0.05;\ \mathrm{M} = 0.08; \mathrm{DP} = 0.119)$ e nAbs $(\mathrm{U}(45) = 189.000; \mathrm{p} < 0.05;\ \mathrm{M} = 0.02; \mathrm{DP} = 0.045)$ para os anticorpos monoclonais. Relativamente ao método de administração, os medicamentos com administração intravítrea apresentam uma diferença estatisticamente significativa para ADAs $(\mathrm{H}(45) = 11.078; \mathrm{p} < 0.05); \mathrm{M} = 0.21; \mathrm{DP} = 0.082$, e a administração intravenosa para nAbs $(\mathrm{H}(45) = 7.073; \mathrm{p} < 0.05); \mathrm{M} = 0.02; \mathrm{DP} = 0.056$.

Quanto ao grupo com atividade enzimática ou reguladora (grupo 2), as enzimas e hormonas apresentaram diferenças estatisticamente significativas para os ADAs $(\mathrm{H}(33) = 43.950; \mathrm{p} < 0.05); \mathrm{M} = 0.20; \mathrm{DP} = 0.252$, bem como a administração subcutânea $(\mathrm{H}(33) = 43.950; \mathrm{p} < 0.05);\ \mathrm{M} = 0.10; \mathrm{DP} = 0.265$. Não foram identificadas diferenças significativas para os nAbs neste grupo.

Foi notificado um total de 47 reações adversas para medicamentos do grupo 1 e 31 para medicamentos do grupo 2. As reações adversas imunogénicas notificadas com mais frequência para o grupo 1 foram reações no local de injeção, anafilaxia, hipersensibilidade e pirexia. Para medicamentos administrados por via intravenosa e subcutânea, a reação adversa mais frequente foi anafilaxia. Para os medicamentos do grupo 2 a hipersensibilidade e as reações anafiláticas foram as que apresentaram maior frequência. As reações de hipersensibilidade parecem estar mais relacionadas com a administração intravenosa enquanto a administração subcutânea está mais frequentemente associada a reações anafiláticas.

Embora os Relatórios Públicos de Avaliação de medicamentos aprovados mais recentemente apresentem um maior número de dados relativos à imunogenicidade, continua a ser necessário harmonizar estes resumos, uma vez que a informação não é apresentada da mesma forma para todos os medicamentos. Deve ser dada mais atenção às hormonas e enzimas, uma vez que não foram detetados tanto ADAs como nAbs durante os estudos de imunogenicidade efetuados durante os ensaios clínicos. Além disso, relativamente às atividades de farmacovigilância pós-comercialização, devem ser feitos mais esforços por profissionais de saúde e pacientes para melhor identificar o medicamento biológico assim como o número de lote.

Palavras-chave: medicamentos biológicos; avaliação regulamentar; imunogenicidade; ADA; nAb; método de administração; reações adversas imunogénicas.

Abstract

The regulatory activities are crucial in the evaluation of the efficacy and safety of biological medicines. Due to their origin and mode of action, immunogenic reactions are more likely to occur when biological medicines are used. Therefore, in order to ensure patient safety, it is extremely important to develop adequate immunogenic studies. Both Food and Drug Administration and European Medicines Agency issued guidelines that aim to assist Marketing Authorisation Holders to develop immunogenicity studies, being, in general, its methodology similar. The type of therapeutic protein and method of administration may influence the emergence of Anti-drug antibodies (ADAs) and/or neutralizing antibodies (nAbs) which can trigger immunogenic responses.

For the biologicals with targeting activity (group 1), higher levels of ADAs ($U(45) = 154.000; p < 0.05;\ M = 0.08; SD = 0.119$) and nAbs ($U(45) = 189.000; p < 0.05;\ M = 0.02; SD = 0.045$) were detected for mAbs. Regarding the method of administration, medicinal products with intravitreal administration showed a significant difference for ADAs ($H(45) = 11.078; p < 0.05); M = 0.21; SD = 0.082$, and intravenous administration for nAbs ($H(45) = 7.073; p < 0.05); M = 0.02; SD = 0.056$.

As regards to the group with enzymatic or regulatory activity (group 2), the products enzymes and hormones showed significant differences for ADAs ($H(33) = 43.950; p < 0.05;\ M = 0.20; SD = 0.252$) as well as the subcutaneous administration ($H(33) = 43.950; p < 0.05);\ M = 0.10; SD = 0.265$. No significant differences were identified for nAbs within this group.

A total of 47 possible adverse reactions were reported for medicines with targeting activity and 31 for medicines with enzymatic or regulatory activity. The most frequent adverse reactions reported for the System Organ Classes (SOC) “Immune system disorders” within the

first group were injection site reactions, anaphylaxis, hypersensitivity, and pyrexia. For medicines administered intravenously and subcutaneously the most frequent adverse reaction was anaphylaxis. For medicines belonging to the group with enzymatic or regulatory activity, the hypersensitivity and anaphylactic reactions were the ones with higher frequency. Hypersensitivity reactions seem to be more related with intravenous administration while subcutaneous administration is more commonly associated with anaphylactic reactions.

Although the most recent European Public Assessment Reports have the information well presented, MAHs need to harmonize the summaries of immunogenicity, since the information is not presented in the same manner for all medicines (period of sampling, preexisting antibodies, placebo and active control group), and for some of the medicine's immunogenicity studies have not even been carried out. More attention should be given to medicines such as hormones and enzymes since most of these products have not detected neither ADAs nor nAbs. Additionally, regarding post-marketing pharmacovigilance activities, more efforts should be carried out by healthcare professionals and patients to better identify the biological medicinal product and batch number.

Keywords: biological medicines; regulatory assessment; immunogenicity; ADA; nAb; method of administration; immune adverse events.

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To my coordinator for guiding me throughout this process.

List of abbreviations

ADA – Anti-drug Antibody

ADRs – Adverse Drug Reactions

BLA- Biologic License Application

CBER- Centre for Biologics Evaluation and Research

CHMP- Committee of Human Medicinal Products

EEA - European Economic Area

EMA- European Medicines Agency

EPAR – European Public Assessment Report

EPO - Epoetin

FAb – Fragment Antibody Proteins

FcFP - Fc Fusion Protein

FDA- Food & Drug Administration

G-CSF - Granulocyte Colony-Stimulating Factor

GH – Growth Hormone

GM-CSF - Granulocyte-Macrophage Colony-Stimulating Factor

GMP –Good Manufacturing Practices

GVP – Good Pharmacovigilance Practices

HCPs – Health Care Professionals

ICH - International Council for Harmonisation

IFN – Interferon

Ig - Immunoglobulin

IGF-1 - Insulin-like Growth Factor-1

IND- Investigational New Drug

IRB- Institutional Review Boards

MAA- Marketing Authorisation Application

mAb – Monoclonal Antibody

NABP - Non-antibody Binding Proteins

NCL- National Control Laboratory

NRA- National Regulatory Agency

PHS- Public Health Service

PK – Pharmacokinetic

PSUR - Periodic Safety Update Report

rhGH – Recombinant Human Growth Hormone

RMP – Risk Management Plan

rTPA – Recombinant Tissue Plasminogen Activator

SmPC – Summary of Product Characteristics

Treg – Regulatory T cell

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1 Introduction

1.1 Biological Medicines

Biological medicines have been produced since the early 1980s, and their importance has been highly recognized, considering their ability to access health areas which had a pharmaceutical gap in the past. (ICH, 2008)

A biological medicinal product is defined by the European legislation as a product the active substance of which is a biological substance, with the biological substance being defined as a substance that is produced by or extracted from a biological source, and that needs for its characterisation and the determination of its quality a combination of physico-chemical-biological testing, together with the production process and its control.(European Commission, 2000)

Biological medicines can be included in numerous categories including serums, toxins, antitoxins, vaccines, blood, or products derived from blood, and can encompass recombinant single protein products, recombinant interferons, monoclonal antibodies, gene therapy vectors, cell therapies, and subunit or vectored vaccines. (EUPATI, 2015) This class of therapeutic medicines can be comprised of sugars, proteins, and nucleic acids or by a combination of them. Its components can derive from humans, animals or microorganisms using biotechnology methods or other cutting-edge technologies. ("What Are 'Biologics' Questions and Answers | FDA," 2018)

Although, many types of biological medicines exist, throughout this dissertation, it will be mainly discussed biologicals in which the active substances are mainly constituted by proteins and peptides, its derivatives or products in which they are components. It is important to refer that biological medicinal products such as allergens, blood, blood derivatives and vaccines are regulated by a slightly distinct regulatory scheme (Sheets, 2017) and for this reason, will not be further discussed herein.

Biological medicines are very distinct from traditional chemical molecules, particularly as regards to their structure. Considering their characteristics and properties, biological products go through much more complex processes during their manufacturing, including transcription, translation, post-translation modifications and protein folding, reasons why products like these display a much more complex molecule when compared to an average chemical synthesized one. As far as the complexity of a biological molecule goes, it is far superior in size and comprehends an inherent heterogeneous structure. The glycosylation of biologicals also adds a further stride towards their complexity and potential heterogenicity. (Roberts & Gibb, 2013) The heterogenicity of biologicals can be mainly related with their manufacturing processes since they are produced in amalgamate cell systems with different fermentation media and operating conditions. (Morrow & Felcone, 2004)

Additionally, most biological medicines are restricted to extracellular and cell surface molecules in contrast to chemical molecules which can take action within the intracellular environment. (Roberts & Gibb, 2013) The above differences can justify the distinction between these products regarding their development, evaluation and regulatory processes. Both scientific principles and regulatory procedures have been to be adapted, to meet the high quality, safety and efficacy requirements of these molecules. (Sheets, 2017)(Morrow & Felcone, 2004)

The development of biological products like hormones, interferons, interleukins and monoclonal antibodies, had a great advance over the last years, since they enabled the treatment of an array of diseases with an unmet need at the time of their development, such as diabetes, growth problems, cancer and immunological diseases. (Sheets, 2017)

There are about two general categories for therapeutic proteins, one with enzymatic or regulatory function and other with targeting or regulatory activity. Therapeutic proteins with enzymatic or regulatory function work by replacing a protein deficiency, augmenting an existing pathway or providing a new function or activity. Therapeutic proteins with targeting

activities are capable of interfering with the pharmacological activity of molecules or with a pathogenic organism. These proteins may also support the delivery of pharmacologically active compounds or proteins. (Roberts & Gibb, 2013)

Table 1 presents the different types of therapeutic proteins, their functions as well as some examples of approved biological medicines within each category.

Table 1- Different types of therapeutic proteins regarding the groups with targeting or enzymatic or regulatory activity. Adapted from: (Roberts & Gibb, 2003 Introduction to Biological and Small Molecule Drug Research and Development: Theory and Case Studies)

Enzymatic or Regulatory Activity	
Therapeutic Protein	Function
Insulin	Recombinant protein which mimics an endogenous beta cell-derived protein.
IFN	Augment existing inflammatory pathways, work as integral parts of the immune response, have natural immunomodulatory, antiviral and antitumour activities. Grouped into three classes, based on their receptors: IFN-β - reduces T-cell migration to the central nervous system; IFN-α – improves viral clearance; IFN-γ - normalize osteoclast function and stimulate osteoclasts to generate superoxide.
EPO	Growth factor responsible for the development of red blood cells from bone marrow-derived precursors. Aids the treatment of low red blood cell counts due to treatment of cancer produced anaemia and late stages of renal disease.

G-CSF / GM-CSF	Cytokines are responsible for the differentiation of neutrophils, basophils, eosinophils and macrophages. GM-CSF regulates the differentiation of myeloid progenitor cells to granulocyte/macrophage progenitor cells. G-CSF also further differentiates the granulocyte/macrophage progenitors to neutrophils and is essential in maintaining adequate neutrophil levels which are critical for preventing bacterial and fungal infections. GM-CSF functions just upstream of G-CSF.
Growth Factors	Developed for treating disease through the regulation of cell differentiation or survival. Growth factors can include a vast selection of therapeutic proteins, such as rhGH, standard care for GH deficiency indications. IGF-1 is synthesized in the liver in response to GH, and also leads to normal growth.
Coagulation and fibrinolytic regulation	Rebalance haemostasis through coagulation and fibrinolytic processes. In this class are included coagulation factors, fibrinolytic and rTPAs.
Therapeutic Enzymes	Enzymatic replacement for patients who lack some of these activities due to genetic defects. For this reason, mostly target orphan diseases. Some of the indications for these therapeutic enzymes include Gaucher, Pompe and Fabry diseases, Hunter and Maroteaux-Lamy syndrome.
Peptide Therapeutics	Diverse class of compounds and regulated as protein biologicals when comprising 100 or more amino acid residues.

Targeting Activity	
Therapeutic Protein	Function
Monoclonal Antibodies (mAbs)	Multifunctional, identical immunoglobulins. Therapeutic mAbs can work similarly to our natural immune system to fight diseases. mAbs can be divided into different categories such as IgG, Antibody conjugates and bispecific antibodies.
FcFP	Contemplate fusion proteins and are composed of a protein or peptide fused to an IgG Fc domain.
FAb	Engineered single protein antibodies which are involved in stable protein interactions.
NABP	Capable of addressing some limitations of the therapeutic antibodies. Defined as binding proteins or peptides which do not contain an antibody variable region domain to drive the targeting interaction.

The table above displays a vast array of therapeutic proteins categorized according to Leader et al. into two large groups. Within these categories, many therapeutic proteins can be evaluated interchangeably, for example, rTPA is evaluated as an enzyme, however, and considering the examples mentioned above, since they relate with a mechanism of action of maintenance of haemostasis, rTPA could also be considered within the group "Coagulation and fibrinolytic regulation". (Roberts & Gibb, 2013)

Peptide therapeutics present many advantages associated with their small size and flexible structure since these characteristics allow therapeutic peptides to bind to targets inaccessible to larger protein therapeutic molecules. These molecules also show high efficacy, low off-target

related toxicities due to high specific binding and increased tissue penetration for improved target access. (Roberts & Gibb, 2013)

Monoclonal antibodies are by far the most promising protein therapeutics in the market regarding their ability to model the immune system response. These compounds display in their structure two identical heavy and light chains. The N-terminal presents a high variation in the amino acid sequence. These characteristics distinguish the VC region (variable region) in their structure and functionality. Monoclonal antibodies also contain an LC region (constant region) whico is responsible for the identification of an antibody molecule to an Ig class. The Fc domain dictates the structure of the "tail" of the molecule and defines the binding to Fc receptors. This domain is responsible for the interaction with the immune system, and its engineering enabled the refinement of the specificity and affinity of these molecules to desired targets and diverse therapeutic modes of action. (Roberts & Gibb, 2013)

Fc Fusion Proteins contemplate a peptide or protein fused into an IgG Fc domain and can provide half-life extension to smaller proteins, their domains and peptides. Most of these therapeutic proteins have a half-life time estimated between 4 to 17 days and can be designed within different modes of activity. (Roberts & Gibb, 2013)

The development of biological medicines revolutionized the approach to a variety of conditions and brought hope to many patients who lacked specialized treatment. However, these advances translated into major costs and new challenges to the life-cycle management of these medicinal products. (Institute, 2016)

Biological medicines are much more expensive to manufacture and therefore their use is associated to a high-cost burden for healthcare systems and patients as well. For this reason, a high demand respecting the development of biosimilar products was set in motion. A biosimilar is a biological medicinal product that contains a version of the active substance of an already authorised reference product in the EEA, and which has shown similarity to the reference

product in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise. (Medicines Agency, 2016)

Thus, biosimilars are medicines which can treat the same indications and show the same efficacy and safety during the treatment as a biological medicine would, with a considerably smaller cost. Nowadays, a substantial number of biosimilars have been developed, however, the use of this type of product has raised many concerns regarding its efficacy, safety and interchangeability with the correspondent biological medicine. (Sheets, 2017) Although at the beginning of their commercialization the safety of biosimilars was questioned, today many studies have shown that there are no significant differences between biological medicines and biossimilars, for example, in the occurrence of adverse events.

1.2- Immunogenicity of biological medicines

The immunogenicity of biological medicines is defined by the capacity to elicit an unwanted immune response, accounting for a challenging and important risk regarding the safety of therapeutic proteins and often relating to serious side effects and life-threatening immunogenic adverse reactions. There are many reasons linked to the emergence of unwanted immunogenic responses to the therapeutic agent, and for this reason, a variety of factors must be taken into account during its development, to assess its possible risks and manage its occurrence. The immunogenic reactions caused by biological medicines can impact the clearance rate, and the efficacy and safety of the therapeutic agent. (Roberts & Gibb, 2013) (Faraji et al., 2018) (Boehncke & Brembilla, 2018)

The immunogenic response triggered by biological medicines mainly happens due to two possible mechanisms: activation of an adaptive immune response to non-self-epitopes on the drug or the loss of immune tolerance. Humoral responses to biological medicines are usually related with an adaptive response to foreign antigens, leading to the expansion of memory T-cells (and adaptive regulatory T cells), and B cells specific to foreign proteins. (Vultaggio et al., 2016)

The mechanisms that underlay the activation of B cells can occur in a T-cell dependent or independent process. For humoral responses independent from T-cells, typically a sequence in the biological medicine induces signals that stimulate B-cells and in most cases do not lead to maturation or generation of memory T cells. However, T-cell dependent humoral responses, which activate B cells, enable a more robust immune response, with an isotype switch (immunoglobulin class switching, for instance from an IgM to an IgG) and memory B cells. Taking this into consideration, to generate memory B cells and induce ADA formation, T-cells must recognise biological peptides. (Vultaggio et al., 2016)

There are two types of anti-drug antibodies (ADAs), the non-neutralizing ADAs, which will be mentioned in this dissertation just as ADAs and the neutralizing ADAs from here on, mentioned as nAbs. The latter, bind to epitopes within the antigen-binding sites of biological medicines, preventing the binding between the medicine and the therapeutic target and thereby resulting in a loss of efficacy. However, ADAs recognize epitopes away from the antigen-binding site, and for this reason, are not directly related with the loss of efficacy of biological medicines, but instead are still of clinical relevance since they can impact the medicine pharmacokinetics. (Boehncke & Brembilla, 2018)

One of the reasons why immunogenic reactions are more common in therapeutic proteins lays on the difference in size and structure of these molecules when compared to a chemically derived drug. Due to its size, almost all biological medicines have to be administered parenterally, since other administration methods, for instance oral administration, would not be feasible for biologicals, mainly because biological medicinal products show lower stability and greater sensibility to enzymatic degradation. (Roberts & Gibb, 2013) Parenteral administration can lead to serious adverse events such as protein hypersensitivity, pain, and toxic reactions. When comparing the adverse reactions caused by therapeutic proteins with chemical substances, their onset is much slower in the later and for this reason, it is extremely necessary to develop faster interventions regarding adverse events related to therapeutic proteins when comparing to other medicinal products administrated through oral or cutaneous route. The administration of therapeutic proteins through parenteral routes enables therapeutic proteins reaching the necessary therapeutic concentration, however, its production is expensive, allows for higher risk of infections and the method of administration can be uncomfortable for many patients, although the latter is not specific to this type of medicinal products. The immunogenicity associated with these products is higher when administered subcutaneously than by intravenous administration, mainly due to the anatomy of the skin and subcutaneous

space, and the existence of dendritic cells in these structures. (Bouwman-Boer, 2015)(Fathallah, Bankert, & Balu-Iyer, 2013)

Another factor associated with the immunogenicity of therapeutic proteins is based on their origins, that is to say, that it was thought that these reactions were mainly related to the host-cells used to create therapeutic proteins. Biological medicines who were developed using non-human cell cultures, e.g. mouse-derived, do have indeed more chances of triggering immune responses when compared to chimeric or fully human cell cultures. This is mostly because glycosylation is species and cell-specific, and depends on cell culture conditions, it is likely that the endogenous and recombinant proteins exhibit different glycosylation patterns. Consequently, antibodies induced by one product may or may not cross-react with another product. (CHMP, 2008) For these reason, the change of the manufacture of some biological medicines to fully-human cell cultures has helped contain the occurrence of some immunogenic responses, however, patients still develop immunogenic responses. (Bouwman-Boer, 2015)(Roberts & Gibb, 2013)

The mode of action of the therapeutic proteins can also determine if they are more or less prone to develop an immunogenic response. Studies show that the immunogenicity of these medicinal products is exacerbated with the use of replacement proteins when compared with binding therapeutic proteins such as mAbs, FcFCPs and NABPs. (Roberts & Gibb, 2013) Treatments which employ replacement therapeutic proteins can trigger an immune reaction by the body and can result in an immune-mediated clearance of the intrinsic protein available. In these situations, besides the clearance of the administered therapeutic protein, the body also eliminates its proteins and lead to the development of severe medical conditions. On the other hand, binding therapeutic proteins are mostly related to higher clearance rates, which limits the exposure to the treatment and reduces its efficacy. (Roberts & Gibb, 2013)

Besides the above factors, other reasons are also associated with the immunogenicity of biological medicines such as the dosing regimen, type of disease, treatment duration and frequency, impurities, patient features (gene factors, age and disease related factors) and other unknown factors. (Crommelin, Sindelar, & Meibohm, 2013)

The occurrence of immunogenic reactions according to the dose regimen is somehow well established. Higher dose treatments are usually associated with lower ADA frequencies. For some indications, in well-established diseases mainly treated with biological medicines, for example, rheumatoid arthritis, both EMA and FDA recommend the use of concomitant medicines, such as methotrexate, to achieve lower ADA incidence. FDA's guidances also state that the intermittent use of biological medicines is more immunogenic when compared to continuous higher dose administration. (Boehncke & Brembilla, 2018)

To reduce the immunogenicity of biological medicines, many approaches have been taken by the industry, such as the biotechnology engineering of these molecules but also the development of new structures and molecules which reduce the possibility of immunogenic reactions. Therefore, it is extremely important to recognise which factors take part in the immunogenicity of these medicinal products, so it can be overcome by the use of new strategies. Most decisions taken during clinical practice are empirical, and for this reason, the shift to an evidence-based strategy is extremely important. (Garcês & Demengeot, 2017)

The assessment of potential immunogenicity of biological medicines and decrease of clinical consequences undergoes the measurement of ADA and nAb formation. For this reason, immunoassays represent a common procedure to detect ADA and nAb presence, and the detection system can be based in various formats, such as direct, indirect, bridging and competitive platforms using radioligand, enzymatic, fluorescent, chemiluminescent or electrochemical luminescence as the detection system. The problem related with the use of these assays lays on the fact that many of these tests are not specific enough to detect ADAs in the

presence of the therapeutic medicine, and this limitation is often referred to as drug interference. (Garcês & Demengeot, 2017)

In order to ensure the safety of the therapeutic protein and the effective patient care as well as disease management , the chosen immunoassay should be drug-tolerant and the characterization of relevant ADAs should support clinical options. (Tatarewicz et al., 2014)

1.3- Regulatory principles in the European Union and the United States of America

Regulatory schemes play a crucial role in the development and evaluation of biological medicines and such activities are conducted since the early stages of the development of these medicines and continue throughout their life-cycle management.

To assess possible problems related with the development of new medicinal products and to ensure that every medicinal product marketed is associated with high standards regarding its efficacy and safety, regulatory agencies have developed legal documents and guidelines which support manufacturers and marketing authorisation applicants and holders to guarantee compliance with such high standards. Also, the regulatory science behind biological medicines is extremely important in protecting public health, patients and HCPs.

The Food and Drug Administration (FDA) and European Medicines Agency (EMA) are governmental agencies responsible for the regulatory activities carried out in the United States of America and in the European Union/ European Economic Area, respectively. These agencies are responsible for the elaboration of a system which enables the licensing of all biological medicines under their jurisdiction and also work on standardizing and controlling all activities regarding therapeutic proteins. (Kurki, 2019)

Nowadays, the FDA acts as a National Regulatory Agency (NRA) and has the responsibility to regulate biological medicines in the United States. Due to FDA's duties, it was necessary to fragment it in National Control Laboratories (NCLs), such as the Center for Biologic Evaluation and Research (CBER), which has the responsibility to protect and enhance public health through the regulation and evaluation of biological medicines in the United States. (Sheets, 2017)

The Center for Biologic Evaluation and Research (CBER) evaluates the scientific data submitted by the marketing authorisation applicant and determines whether this data comply or

not with the set standards, but also generates an evaluation based on a risk-benefit assessment. Thus, CBER is committed to approve medicines that can prove to maximize the benefits and minimize the risks to patients treated with these types of medicines. ("About CBER | FDA," n.d.)

The legal requirements set for biological medicines can be found in the Code of Federal Regulations Title 21, also known as 21 CFR, through articles 600 to 660. Besides the articles meant for the regulation of biological medicines, it is also necessary to act accordingly to the Current Good Manufacturing Practices (GMP), set in Titles 210 and 211, Institutional Review Boards (IRB) regulations set in Titles 50, 56 and 58, and Investigational New Drugs (IND) regulations set in Title 312, where applicable.

The European Medicines Agency, as mentioned above, is the European governmental agency responsible for the regulation of medicines in the European Union. Although EMA is responsible for the evaluation of the biological medicines evaluated through the centralized procedure, there are other 31 EEA countries (28 EU Member States, plus Iceland, Liechtenstein and Norway) national agencies responsible for the evaluation of biologic medicines submitted through other procedures. The Committee for Medicinal Products for Human Use (CHMP) is the committee within EMA responsible for the evaluation and regulation of human medicines. The general requirements for biological medicines are set in the Directive 2001/83/EC of the European Parliament and of the Council and are also supported by other documents such as the European GMP guideline. In Europe, biological medicines are largely defined in terms of their active substances and manufacturing methods. (Kingham, Klasa, & Carver, 2013)

Starting with the definition of biological medicine, as previously mentioned, the above mentioned directive defines this class of medicines as "product(s), the active substance of which is a biological substance", the latter defined as a "substance that is produced by or extracted from a biological source and that needs for its characterization and the determination of its

quality a combination of physicochemical-biological testing, together with the production process and its control". For FDA, a biological medicine is defined as "any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment or cure of diseases or injuries of a man". (Kingham et al., 2013)

The definition of biological medicine in the United States has been evolving through time and shaped while accompanying the development of arising therapeutic proteins. On the other hand, in the European Union/ European Economic Area, this definition is considered to be more wide-ranged and generic but has not evolved over time. (Kingham et al., 2013)

To license a biological medicine in the United States, developers need to submit a BLA, process differentiated from the licensing of a chemically derived medicine. However, in Europe, EMA regulates biological medicines in the same general authorisation/regulatory scheme as for chemically derived substances. (Kingham et al., 2013)

Biological medicines are indeed regulated in the USA and the EU/EEA within different legislations and regulatory philosophies, but the strategies defined by FDA and EMA are considered to be overlapping. Both recognize the importance regarding the regulation of these medicinal products, due to their differences to other chemically derived medicines, and also recognize the importance of the immunogenicity inherent to all medicines of this class. (Kurki, 2019)(Kingham et al., 2013)

Until 1997, the regulation of biological medicinal products was divergent, both in the United States and in Europe, since both applicable legislations had no connection whatsoever with each other. At this point, the International Council for Harmonisation (ICH) developed the ICH S6 guideline "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" intending to allow for a better understanding, evaluation and preclinical development of these medicines. Since both EMA and FDA adopted this guideline, the regulation of biological

medicines became more convergent. (Kingham et al., 2013) This guideline did not have the purpose to endorse law changes, regulations or issued guidelines. (ICH, 1995)

However, after the adoption of the ICH S6 guideline a disharmony across regions was still noticeable due to differences in the its implementation and. For this reason, an addendum was proposed to clarify five topics of the guideline such as species selection, study design, immunogenicity, reproductive and developmental toxicity and assessment of carcinogenic potential. The CHMP adopted the guideline ICH S6 (R1), in July 2011 and the FDA in May 2012. (ICH, 2008)

After the FDA has adopted the ICH S6 guideline, it has also issued a new and developed Guidance for Industry "Immunogenicity Assessment for Therapeutic Protein Products" which reflects the actual thinking of the FDA and is meant to serve as a recommendation for the industry. Manufacturers and marketing authorisation applicants are allowed to develop other in-house assays, besides the ones set in this guidance but in these cases they must assess, evaluate and prove the reliability of these procedures, considering the accuracy and robustness of the new methods. These Guidances should only be seen as recommendations, and do not establish legally enforceable responsibilities. (US Department of Health and Human Services, 2011) The same applies to the guidelines issued by the International Council for Harmonization (ICH) and adopted by the CHMP.

The guidelines issued by these agencies advise on the methodology and approaches that the Marketing Authorisation Application (MAA) should address when developing a new biological product and further when submitting a marketing authorization application.

To compare the methodology concerning the assessment of the immunogenicity of biological medicines both documents published by these agencies will be analysed below.

Table 2 summarizes the differences and similarities between EMA's Guideline on "Immunogenicity assessment of therapeutic proteins" (EMEA/ CHMP /BMWP/ 14327/ 2006

Rev 1) and FDA's Guidance for Industry "Immunogenicity Assessment for Therapeutic Protein Products" when assessing the possible immunogenicity of biological medicines.

Table 2 - Comparison between FDA's Guidance for Industry "Immunogenicity Assessment for Therapeutic Protein Products" and EMA's Guideline on "Immunogenicity assessment of therapeutic proteins" (EMEA/ CHMP /BMWP/ 14327/ 2006 Rev 1) and when assessing the potential immunogenicity of biological medicines Adapted from: (Kurki, 2019 Compatibility of immunogenicity guidance by the EMA and the US FDA).

Guideline/Guidances Topics	**Regulatory Agency Guidance**	
	FDA	**EMA**
The strategy of immunogenicity studies	Highlight the importance of immunogenicity studies and suggest a risk-based approach to this matter. Also emphasize the importance of developing a risk assessment and a thorough analysis of a specific product, as well as to patients and disease-related factors that can trigger harmful immune responses. However, tests for determination of T cells immunity, such as ELISPOT, are not usually required. (Bercovici, Duffour, Agrawal, Salcedo, & Abastado, 2000) Both agencies believe that ADA and nAb detection should be accomplished. Developers shall establish studies about cross-reactions of the ADAs against a biological product with corresponding endogenous proteins.	
Timing of assay development	All assay strategy and development shall be designed before clinical development. These data must be presented together with the marketing authorization application.	
The sensitivity of ADA assays	1. Recommends that screening and confirmatory assay achieve a sensitivity	1. Has not defined a target sensitivity value.

Guideline/Guidances Topics	Regulatory Agency Guidance	
	FDA	**EMA**
	of at least 100 nanograms/mL. 2. FDA accepts up to 1% false positives.	2. For the confirmatory assay, EMA expects no false positives.
	Besides above-shown differences, both agencies agree that the assays used should be sensitive for relevant ADA isotypes and capable of handling a large volume of samples. It is also important that these assays are capable of detecting low levels of ADAs before its concentration reaches higher levels, which are capable of altering pharmacokinetic, pharmacodynamic, safety and efficacy of the therapeutic proteins. For the confirmatory assay, MAAs should use purified animal ADAs or a mixture of human mAbs.	
<u>Medicine Interference</u>	Recommends sponsors to evaluate the drug tolerance of the assay developed, in the early stages of the essay planning.	Defends that the drug tolerance of the developed assay must be higher than the expected drug levels in the clinical samples.
	Both recommend sampling for ADAs at the time when the concentration of the therapeutic protein is considered the lowest.	

Guideline/Guidances Topics	Regulatory Agency Guidance	
	FDA	**EMA**
Neutralization Assays	Consider the evaluation of neutralizing ADAs very important when assessing the immunogenicity of biological medicines. There are two possible assays for detecting nAbs, cell-based and binding and non-cell-based assays.	
Role of nonclinical studies	Nonclinical studies are important to assess dose-repeated pharmacological and toxicological studies. Besides these studies, both agencies agree that nonclinical studies and the use of novel *in vivo*, *in vitro* and *silico* approaches are not considered to be sufficiently predictive of the immunogenicity of medicines in humans but consider them to be useful in describing the consequences of antibody responses.	
	FDA has not specified if any measures will be taken to reduce animal testing.	UE is collecting efforts to reduce animal testing.
Clinical immunogenicity studies	Aims to investigate antibody immune reactions and their severity. Allows a shorter pre-marketing authorisation follow-up period for medicines intended for	Stands by the idea that immunogenicity studies aim to detect and characterize an immune response to a biological product by correlating the presence of ADAs with the

Guideline/Guidances Topics	Regulatory Agency Guidance	
	FDA	**EMA**
	chronic administration if properly justified.	pharmacokinetic, pharmacodynamic, efficacy and safety of the product.
	It is mandatory to assess immunogenicity in all pivotal clinical studies in a targeting population who has not been previously exposed to medicine. This evaluation should be done on a case-by-case basis, and its extent should be based on the probability of the occurrence of immunogenic events and the potential severity of those events. The duration of the follow-up should also be product-related and according with the duration of treatment.	
<u>Sampling of ADAs</u>	Sampling is appropriate during the early stages of exposure, especially if the risk for immunogenicity is high. If adverse events occur and are suspected to be immune-related, unscheduled sampling is recommended. In repeated administration schedules, ADA samples should be drawn before the administration of the medicine. During clinical trials, both agencies suggest real-time assessments, if the situation is considered high-risk, for example when there is a chance for cross-reaction with endogenous proteins.	

Guideline/Guidances Topics	Regulatory Agency Guidance	
	FDA	**EMA**
Immune-Mediated Adverse events	Recommend separate analysis regarding patients with pre-existing antibodies at baseline and exclusion of these patients from the cut point analysis. Clinical development plans should also define key potential immune-mediated events including loss of efficacy.	
	FDA warns that if patients have been priory exposed to a biological medicine, there is a chance for pre-existing antibodies to be detected at baseline.	The severity of immune-mediated adverse events should be considered when evaluating the data. It is extremely important to evaluate possible pathological changes related to immune complex formation and deposition.
Mitigation of adverse immune reactions	Both FDA and EMA stress the importance of exploring mitigating measures in case of immune-mediated adverse events or loss of efficacy.	
	Regarding dose-escalation strategies, these will depend on the product, therapeutic indication(s) and magnitude of the antibody response. Protocols, to overcome	Mitigation measures are often applied during clinical practice. For this reason, EMA regards the development of mitigation measures, however, these

Guideline/Guidances Topics	Regulatory Agency Guidance	
	FDA	**EMA**
	antibodies effects, should encompass stopping rules and safety monitoring measures before proceeding to a dose escalation. Sponsors in some cases are encouraged to explore induction regimens in a prophylactic setting, for situations where patients are treated with a lifesaving therapeutic protein.	are not mandatory before the marketing authorisation approval.
<u>Quality improvement of immunogenicity data</u>	May consider an "Integrated summary of immunogenicity" in its guidance, however, this is not mandatory to obtain a marketing authorization.	Highlights once more the importance of developing product-specific and target population assays. Requests the addition of the "Summary of immunogenicity" in the marketing authorisation request, allowing MAAs to justify their risk-based approach.

Guideline/Guidances Topics	Regulatory Agency Guidance	
	FDA	**EMA**
Post-marketing assessment of immunogenicity	Encourages sponsor to request for guidance, by consulting with the agency since it will allow better planning of the approach to post-marketing safety monitoring. FDA also states that sponsors shall consult with the agency regarding post-marketing sampling of ADA outside clinical trials. To monitor potential adverse effects at this phase the database "Sentinel Initiative" was created.	Obliges MAHs to develop a Risk Management Plan (RMP). Often MAHs list immunogenicity as a risk in its RMP.

Both FDA and EMA emphasise that the type and magnitude of an immune response are determined by the host immune system, hence the importance of the development in a case-by-case basis of methods that could evaluate the immunogenicity of biological medicines. Unfortunately, it is still not possible to predict harmful immune responses to its full potential, since many of the mechanisms that trigger these kinds of reactions are still unknown. (Kurki, 2019)

The timing for post-treatment collection of samples for ADA detection is dependent on the half-life of the therapeutic protein, which highlights once more the importance of a case-by-case evaluation and methodology. The ADA assay methodology has evolved over the years and the nowadays used, bridging assays, are more sensitive than the ones used in the past, and evaluate the immunogenic potential more truthfully, however, this type of assays provides less accurate evidence for neutralization ADAs. (Kurki, 2019) The bridging assays develop less accurate evidence for neutralization ADAs, mainly because it is more challenging to develop assays that show direct interference of a biological function. In particular, the need for adequate drug and target tolerance often requires extensive pre-treatment steps that limit assay sensitivity compared with a typical bridging-format assay used to detect binding ADA. (Bercovici et al., 2000)

The Food and Drug Administration guidance also provides more information for the diagnosis and reporting of acute and delayed immune responses, which can be highly valuable to the evaluation of the immunogenic potential of biological medicines, as well as being more explanatory on the subject of mitigation of adverse immune responses and tolerance induction. (Kurki, 2019)

The Guidance for Industry "Immunogenicity Assessment for Therapeutic Protein Products" issued by the FDA is more detailed and explanatory, specially respecting the technical matters of assays for ADAs (for e.g., regarding the sensitivity of the ADA assays), than the ICH S6 (R1) guideline, adopted by both agencies (FDA and EMA). These differences can have some advantages as well as disadvantages. EMA's guideline and regulatory actions allow MAAs to have more freedom when developing their medicinal product and only interferes if the developer requests scientific advice. On the other hand, FDA's guidance is more detailed and gives additional options for developers during the mandatory or requested

meetings, that can sometimes lead to the need of more resources for developers to be able to meet all requested requirements. (Kurki, 2019)

Thorugh my point of view, in the future, both agencies should strengthen the control of ADA assays, since many biological medicines were approved without appropriate or with uncertain information on immunogenicity. Also, the roles of neutralizing assays should be re-evaluated since the benefit-risk assessment is more focused on the ADAs impact in Pharmacokinetics (PK). For this reason, in the presence of relevant clinical data, these assays are questionable because most of the developed assays cannot evaluate the indirect effect of nAbs. In some cases, ADAs against therapeutic proteins are known to be connected in the antigen-binding region, and for these cases, the ADA assays mainly measure the same ADAs as the nAbs assay. It is also important to mention that in most cases if the MAA does not request scientific advice, which can be expensive, if there is any problem regarding the method used for the assays, it will be only detected in a later stage of the application. In such cases, EMA should have requested improved assays, which has not been always the case, since some biological medicines were approved in an environment which was not consistent with the regulatory "rules" set by this agency. (Kurki, 2019)

The European Medicines Agency (EMA) has also issued in 2012 the "Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use" (EMA/ CHMP/ BMWP/ 86289/ 2010. The European agency stresses that this guideline aims to provide further guidance of the immunogenicity assessment only for mAbs, their derivatives, and products of which they are components, e.g., conjugates, Fc linked fusion proteins, which were divergent to the ones set in the general guideline for immunogenicity assessment. Thus, this document further explains some problems experienced with screening and confirmatory assays, the neutralizing capacity of mAbs and explores the actions that should be taken to manage the risk of immunogenic reactions to mAbs. (CHMP, 2012a)

In general, the screening assays used for the detection of ADAs in patients treated with mAbs are more challenging and complex when compared to other therapeutic proteins. For this reason, MAAs must develop assays which are sensitive enough to detect its presence. Since mAbs have long half-life periods and persist on the serum for long periods, sponsors shall consider this fact, and try to examine ADAs samples when there is known to be a lower concentration of therapeutic protein in the blood. The use of protein A or G (used in Radioimmunoprecipitation assays (RIPA)) is considered inappropriate since very often these bind to the product itself, being the bridging format often recommended to detect ADAs to mAbs. (CHMP, 2012a)

Regarding confirmatory assays, it is recommended the used of Proteins A or G, since these confirm that the positive result was due to an immunoglobulin. It is mandatory to evaluate the neutralizing capacity of the mAb, and if not, an explanation should be provided by the MAA. This guideline also emphasizes the approaches to the risk identification and management, which should be also assessed in the Risk Management Plan of the mAb. (CHMP, 2012a)

Based on the regulatory principles/strategies mentioned above, the guidances issued by FDA and EMA regarding the assessment of the immunogenicity of biological medicines, and the general regulatory activities developed by both agencies do not seem to differ and therefore have a major impact on the assessment of immunogenicity of the biological medicines. (Kingham et al., 2013)

Clinical trials play an important role when it comes to the evaluation of the success and safety of the developed biological medicinal product. The basis regarding the conduction of clinical trials lays in the preclinical data collected through this phase of testing. In the United States, FDA requests that the application for the execution of clinical trials must be submitted through an Investigational New Drug (IND) application, where the agency evaluates if the data collected is reasonably safe to approve the clinical trial. In these applications, sponsors must

submit the information that allows studying the safety, but also the chemistry, manufacturing and controls, considering that biological medicines arise particular concerns related to its impurity properties. (Kingham et al., 2013) The FDA bases its verdict in the Good Clinical Practices (GCP) and has also adopted the ICH E6 (R2) guideline "Guideline for Good Clinical Practice". The study design for clinical trials contemplating biological medicines must include an assessment of immunogenicity, in which sponsors must evaluate ADAs directly after administration and at least 28 days thereafter. (Kingham et al., 2013)

Phase I of clinical trials aim to evaluate the maximum tolerated dose and assess the bioactivity of the product, to determine the optimal biological dose. Phase II determines short-term adverse events and the efficacy of the medicinal product and helps to prepare Phase III studies. Phase III studies focus on the evidence for labelling claims and risk-benefit assessment. FDA also stresses the importance of the chosen endpoint, being critical for the success of this phase of the clinical trial. The approval of a biological medicinal product can also be supported by the "animal rule", in which sponsors base their studies on human safety data and adequate controlled animal studies. (Kingham et al., 2013)

In Europe, sponsors base their application for a clinical trial on the requirements set in the Directive 2001/20/EC. Although the Clinical Trial Regulation (EU) No 536/2014 entered into force on 16 June 2014, the timing of its application depends on the development of a fully functional EU clinical trials portal and database. (European Commission, 2018) As well as FDA, in the European Union, national authorities also base the approval of a clinical trial in the GCP as described in Regulation 2005/28/EC and the ICH E6 (R2) guideline "Guideline for Good Clinical Practice". According to this regulation and guideline, respectively, sponsors shall always obtain informed consent and comply with the declaration of Helsinki when conducting the clinical trial. (Kingham et al., 2013)

As for the IND applications in the USA, CHMP has also issued a guideline on quality requirements for Investigational Medicinal Products “Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials”. (CHMP, 2012)

In contrast to the United States, Phase I clinical trials in the EU may be conducted in healthy volunteers. This phase should only recruit patients if the therapeutic protein is considered to be potentially toxic. No differences are found for Phase II and III studies between both regions. (Kingham et al., 2013)

After the TeGenero incident, in which patients experienced severe adverse events including multiorgan failure following a clinical trial with a mAb, CHMP has issued the “Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products” (EMEA/ CHMP/ SWP/ 28367/ 07 Rev. 1) that prioritizes the well-being of the subject enrolled in the study and aims to characterise and manage risk. (CHMP, 2017) (Kingham et al., 2013)

If after the clinical trial an agency grants authorisation, when the animal rule was used, or the medicine was evaluated through an accelerated process, sponsors must continue with post-marketing clinical studies, to guarantee the safety and effectiveness of the therapeutic product.(Kingham et al., 2013)

Both EMA and the FDA adopted the ICH E5 (R1) Guideline “Ethnic Factors in the Acceptability of Foreign Clinical Data”, that aims to facilitate the registration of medicines amongst the ICH regions by recommending the framework for the evaluation of the impact of ethnic factors in medicine. (Kingham et al., 2013) (Kurki, 2019) (ICH, 1998)

Cluster meetings also play a huge role regarding the parallel scientific advice between these two agencies and support an intensified exchange of information and collaboration between EMA and the FDA regarding medicines in which both regions have an interest. (Kurki,

2019) After the parallel scientific advice, both agencies freely publish their own independent opinion on the matter. (Kingham et al., 2013)

1.4. Pharmacovigilance

Pharmacovigilance is defined by EMA as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem". Such activities are meant to be conducted by all MAHs after the approval of the medicinal product and maintained throughout its life-cycle management.

Considering the importance of biological medicines and its immunogenic potential, it is undeniable that pharmacovigilance acts as an essential post-marketing activity for these medicines, since clinical trials enroll a small number of patients, and have a short period of follow-up. For this reason, most of the immunogenic potential of these medicines is detected and evaluated during their post-marketing period and is also crucial for the detection of late-onset adverse events.

Both FDA and EMA recognise the importance of such activities, and to mitigate the risks associated with these treatments, these agencies created pharmacovigilance strategies that should be followed by MAHs.

The Sentinel Initiative, in the United States, aims to create an electronic database in which adverse events can be clustered. This database collects information reported by different sources such as HCPs, insurance companies and health maintenance organisations. (Kurki, 2019)

In Europe, EMA has a different approach to this matter, what can be just which by the different regulatory frameworks within these two regions. As previously mentioned, biological medicines must be approved through a centralized procedure and for this reason, EMA is the agency responsible for the evaluation of the management carried by the MAHs regarding pharmacovigilance activities and its outputs. The pharmacovigilance system is laid down by

the agency in the (EC) No 726/2004, Directive 2001/83/EC and Commission Implementing Regulation (EU) No 520/2012. (Sheets, 2017)

When a biological medicinal product is approved, usually the necessary information regarding its safety has been provided, and proof that the benefits of its use outweighs its risks. However, during clinical practice, other factors take part in the possibility of unwanted and harmful events. The benefit-risk of these medicines was assessed during controlled trials and in most cases without concomitant treatments. This scenario does not replicate the everyday clinical practice and for this reason MAHs in Europe have the legal obligation to monitor and continuously collect data regarding adverse events to their therapeutic product. To better assess the adverse reactions in a real-world context, since 2011 all biological medicines introduced into the market are included in EMA's additional monitoring list. This list aims to identify more rapidly adverse events to new medicines in the market and therefore enhances their reporting. The general principles of the additional monitoring were introduced by the Good Pharmacovigilance Practices (GVP) Module X. (GVP, 2013)

The Pharmacovigilance Risk Assessment Committee (PRAC) was established with the new pharmacovigilance legislation and is responsible for assessing and monitoring safety issues for medicines at the EU level. PRAC also provides recommendations to the CHMP. (Felix et al., 2019)

To better assist MAHs during the pharmacovigilance activities of biological medicines, EMA issued the Guideline for GVP "Product- or Population-Specific Considerations II: Biological medicinal products "(EMA/168402/2014 Corr*). According to this guideline, the main principles of the benefit-risk assessment laid down in the GVP's from Module I to XVI are the same for biological or non-biological medicines. However, it is important to adequate the pharmacovigilance activities to the medicinal product itself, and for this reason, since

biological medicines are considered to have a dynamic quality profile, it is essential to consider aspects such as product traceability during its life-cycle. (European Medicines Agency, 2016)

One of the main concerns for biological medicines is related to changes to the manufacturing process, and for this reason, pharmacovigilance practices as regards to this class of medicinal products should always assess these variations. Manufacturing changes are common and can occur after changes in source materials, facilities, or regulatory requirements. All changes to the manufacturing process of a biological medicine should encompass a comparability proof, to ensure the maintenance of the quality safety and efficacy of the medicine. (European Medicines Agency, 2016)

More than for other medicines, practices such as inadequate storage, handling process, cold chain and GMPs can significantly alter the quality of biologicals, which places more emphasis on the importance of product traceability and batch information. To mitigate the risk associated with these factors, every HCP should handle to the patient the information about the product name and batch code of the prescribed medicinal product. Additionally, the name and batch of the biological medicine should be recorded for traceability purposes, as mentioned in section 4.4 “Special warning and precautions for use” of the Summary of Product Characteristics (SmPC). (European Medicines Agency, 2016)

All biological medicines must have a risk management plan and discuss the immunogenicity potential associated with them, although this plan is not specific to biological medicines. Immunogenicity may occur, but it is not in itself a specific safety concern, and for this reason, MAHs should only include its evaluation if it is classified as an important risk (identified or potential) or if its importance has not been evaluated or there is missing information. (European Medicines Agency, 2016)

The determination of the strategy for the evaluation of immunogenicity should gather different types of information like quality, clinical, non-clinical and pharmacovigilance. A

root-cause analysis should be developed by the MAA to evaluate the ability for risk minimization or elimination encompassing for example optimized manufacturing processes or assays. (European Medicines Agency, 2016)

The RMP shall also include information on the potential of the biological medicinal product to trigger infections caused by residues of biological material or contaminations developed during the current manufacturing process. Proper traceability of the medicinal product also enables the detection of batch-specific issues. (Medicines Agency, 2016)

Periodic Safety Update Reports (PSUR), which support the processes of signal management, should be conducted for biologicals as it is for the other medicinal products. (European Medicines Agency, 2016)

Besides the reporting and signal management activities, safety communication of the adverse reports plays a valuable aspect of pharmacovigilance activities. The communication of the risks associated with biological medicines across all stakeholders, such as HCPs, patients and competent authorities, can define the success of the quality and quantity of gathered adverse events. For this reason, guaranteeing a good understanding of the active substance, its mode of action, excipients and possible residues should be a priority for the pharmaceutical industry during training for HCPs, and by the latter when prescribing to the patient. The demystification of such concepts allows better communication. (European Medicines Agency, 2016)

Similar to the database created in the United States (Sentinel Initiative), all Individual Case Safety Reports (ICSRs) reported within the European Union, either by National Competent Authorities or MAHs are collected in the Eudravigilance Database. These reports aid the evaluation of the benefit-risk profile of the medicines commercialized within the European Economic Area (EEA). To further inform on the suspected adverse effects reported within this space, EMA created a website to share such information (https://www.adrreports.eu/en/).

According to this database, and regarding the biological medicines approved in Europe over the last 10 years, a total of 208 304 adverse events have been reported, of which 15 349 (8%) are related to medicines with enzymatic activity such as growth factors, insulins, hormones, and enzymes, and 192 955 (92%) suspected adverse drug reactions were reported for biological medicines with targeting activity, for instance, monoclonal antibodies and FcFPs.

Figures 1 and 2 summarize the data collected for all biological medicines marketed in Europe in the last 10 years.

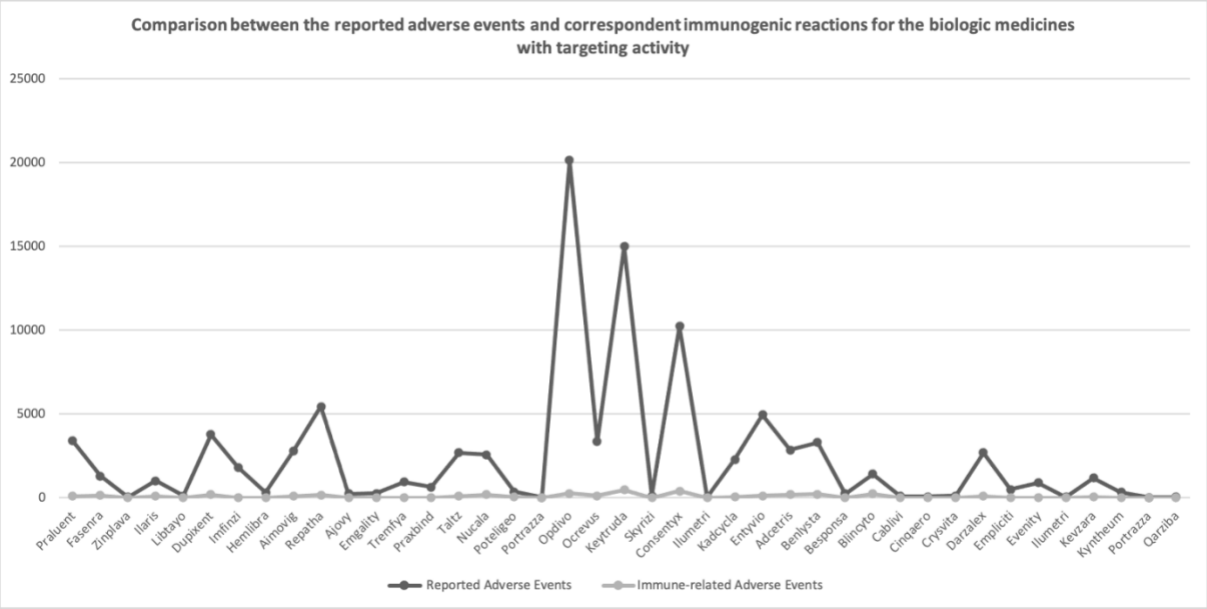

Figure 1- Comparison between the adverse events reported and correspondent immunogenic reactions for biological medicines with targeting activity. Adapted from: https://www.adrreports.eu/en/.

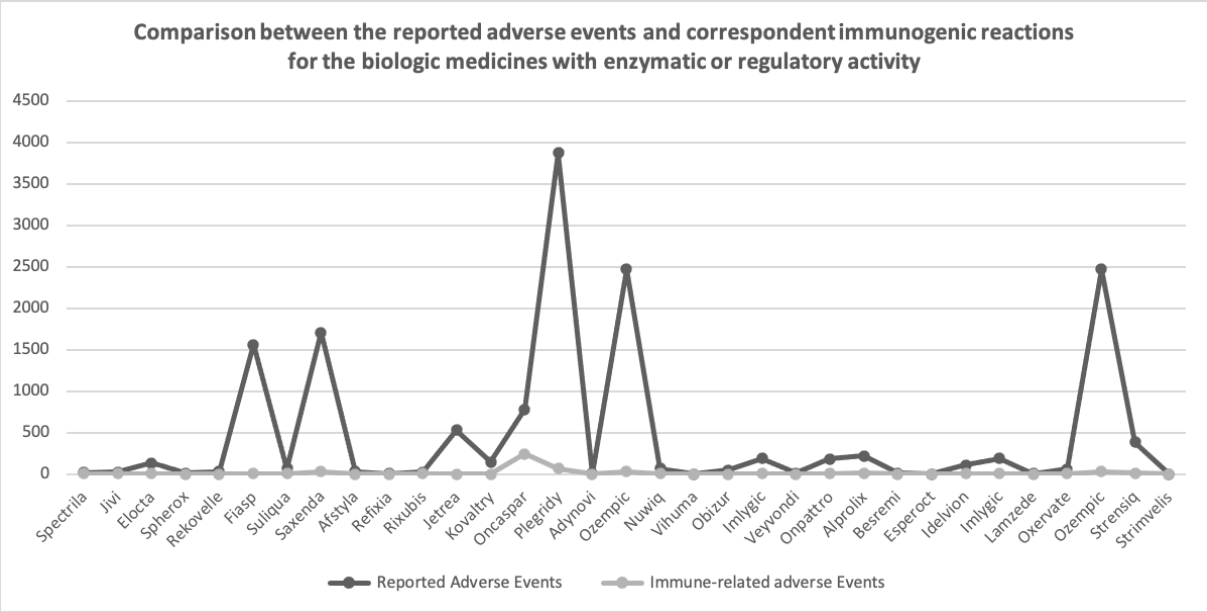

Figure 2- Comparison between the adverse events reported and correspondent immunogenic reactions for biological medicines with enzymatic or regulatory activity - Adapted from: https://www.adrreports.eu/en/

According to the figures above, most of the potential adverse events to biological medicines were not immune-related. The active substances etanercept and pegaspargase, represent the biological medicines from each category with a higher number of immune-related adverse events reported. The immune-related adverse events reported for pegaspargase represent 31% of the total Adverse Drug Reports (ADRs) reported and for etanercept 5.38%. When evaluating the immunogenicity information contemplated in the SmPC of the biological medicine containing the active substance pegaspargase, the information is "No immunogenic response was detected in a 12-week study in mice in which pegaspargase was administered weekly at the dose of 10.5 U/mouse intramuscular or intraperitoneally." Since after its commercialization the percentage of immune-related adverse events related with this active substance is 31%. Although the first data was gathered from animal studies, the information gathered during pre-marketing should be evaluated.

The pharmacovigilance system implemented in the EU has been immensely developed when compared to the practices before the implementation of the 2010 pharmacovigilance legislation. The replacement of a biological reference product for its biosimilar is a common practice, however, it is very important to assure that patients are aware of the change and that the brand, manufacturer or batch number is recorded, since this practice change can lead to ambiguous product information. (Felix et al, 2019)According to a study regarding the report of ADRs in EU to the Eudravigilance database, before the implementation of the new pharmacovigilance legislation, a high amount of reports did not have an identifiable product. (Felix et al., 2019) However, since its implementation, 96% of the reports had an identifiable product name, which raised to 97,2% when the narrative and reporter comments were included. (Felix et al., 2019)

One of the concerns to the actual pharmacovigilance practices is related to the implementation status of the most recent legislation since it can be applied differently within

the Community Member States. Batch traceability also requires improvement which shows that the improved legislation has not yet affected this matter. (Felix et al., 2019)

As a conclusion, the lack of an identifiable product name in pharmacovigilance acts as a delay and overdue the detection of safety signals, however, massive improvements were achieved with the implementation of the pharmacovigilance legislation in 2010. This system adopted product-type-specific pharmacovigilance which should act as a global reference for other health agencies and pertaining regulatory activities. (Felix et al., 2019)

2. Aims

This dissertation aims to evaluate the immunogenicity of biological medicines approved and marketed in Europe in the last 10 years.

To achieve this, the frequency of ADA and nAbs measured during the pre-approval period was used. This frequency was assessed according with the indicated method of administration and also the type of product (this category includes the two main groups of biological medicines - group with targeting activity such as mAb, FcFP, FAb and NABP, and the group with enzymatic or regulatory activity such as insulins, IFN, EPO, G-CSF/GM-CSF, growth factors, biological medicines with coagulation and fibrinolytic function, therapeutic enzymes and peptide therapeutics).

Moreover, all adverse reactions reported during the clinical trials phase and in the context of post-authorisation surveillance and included within the MedDRA SOC (System of Organ Class) “Immune system disorders”, were assessed.

3. Methodology

A retrospective semi-quantitative statistical study was performed.

Study Design

Semi-quantitative statistical studies were performed using SPSS and Microsoft Excel tools. These studies evaluated the frequency of ADAs and nAbs concerning:

- Type of product;
- Method of administration;

Additionally, all adverse reactions related to the SOC "Immune system disorders" were evaluated for both groups of biological medicines and associated with the frequency of ADAs and nAbs.

Setting

Data regarding the frequency of ADAs and nAbs as a response to treatments contemplating biological medicines were retrieved from the European Public Assessment Reports published by EMA on its website. Additionally, the section "4.8 – Undesirable effects" of all SmPCs was evaluated.

Target Population

All patients enrolled during the clinical trial phases of the selected biological medicines, approved in Europe over the last 10 years as well as all patients treated with these medicinal products afterwards.

4. Results and Discussion

Biological medicinal products with targeting activity

Hypothesis 1 - Differences in the frequency of ADAs and nAbs regarding the type of product

Dependent Variable: ADAs and nAbs frequency

Independent Variable: Type of Product (mAb and FcFP)

To evaluate if the variable "type of product" restricts the frequency of ADAs and nAbs, and since the assumption of normality of both samples has not been met, the Non-Parametric Mann-Whitney method was used, because this test aimed to compare two independent variables.

Table 3- Non-Parametric Mann-Whitney (U) of the frequencies of ADAs and nAbs concerning the type of product, for independent samples.

Indexes	Monoclonal Antibody (n = 44)	FcFP (n = 1)	U	p
	M ± SD	M ± SD		
ADAs	0.08 ± 0.119	0.01 ± 0.000	154.	**0.004**
nAbs	0.02 ± 0.045	0.00 ± 0.000	189.	**0.008**

M= mean;

SD=Standard Deviation;

U= Mann-Whitney U test statistics

p= p value

n= number of medicines belonging to each group

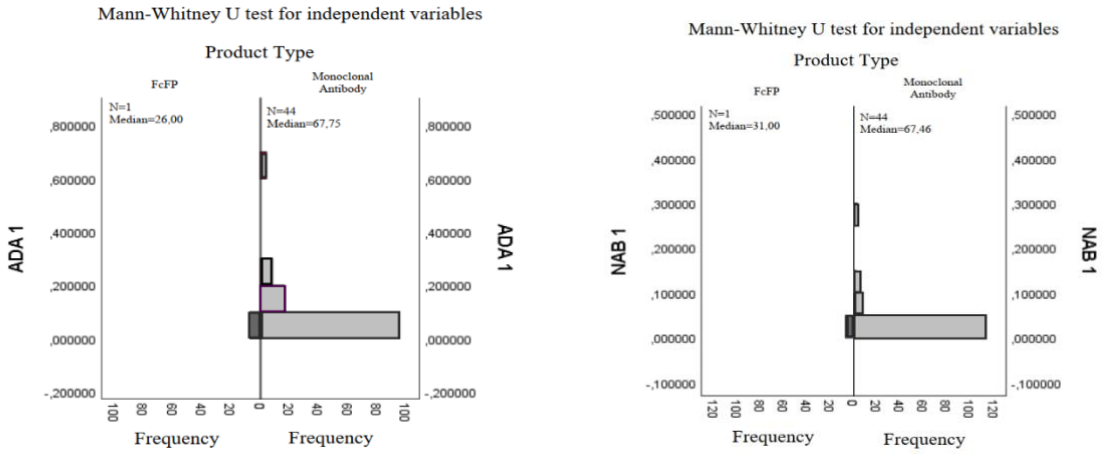

Figure 3 – Mann-Whitney U test for independent variables for ADA and nAb detection regarding the product type.

Thus, as depicted in table 3, statistically significant differences were found between the frequency of ADAs regarding the Product Type $(\mathbf{U(45) = 154.000; p < 0.005})$, in which the monoclonal antibodies present a higher mean than the class FcFP; $\mathbf{M = 0.08; SD = 0.119}$ against $\mathbf{M = 0.01; SD = 0.000.}$

The same happened with the variable nAbs, with statistically significant differences in Product Type $(\mathbf{U(45) = 189.000; p < 0.05})$; also in this case the monoclonal antibody class has a higher mean than the FcFP class $\mathbf{M = 0.02; SD = 0.045}$ against $\mathbf{M = 0.00; SD = 0.000.}$

Hypothesis 2 - Differences in the frequency of ADAs and nAbs regarding the method of administration

Dependent Variable: ADAs and nAbs frequency

Independent Variable: Method of Administration (Subcutaneous, Intravenous and Intravitreal)

To evaluate if the Method of Administration (Subcutaneous, Intravenous and Intravitreal) restricts ADAs and nAbs, and since the assumption of normality of both samples has not been met, the Non-Parametric method of Kruskal-Wallis was used, since the test was performed for three independent variables.

Table 4 - Non-Parametric Kruskal-Wallis Method (H) of the Frequencies of ADAs and nAbs concerning the Method of Administration, for independent samples.

Indexes	Subcutaneous (*n=22*)	Intravenous (*n=22*)	Intravitreal (*n=1*)	H	p
	M ± SD	M ± SD	M ± SD		
ADAs	0.06 ± 0.060	0.08 ± 0.147	0.21 ± 0.082	11.078	**0.004**
nAbs	0.01 ± 0.028	0.02 ± 0.056	0.00 ± 0.000	7.073	**0.029**

M=Mean;

SD=Sdandard Deviation;

H= Kruskal-Wallis H test statistics

p= p value

n= number of medicines belonging to each group

Table 4 presents the statistically significant differences between the indexes ADAs and nAbs regarding the Method of Administration.

Statistically significant differences were identified between the ADAs index and method of administration($\mathbf{H(45) = 11.078; p < 0.05}$), where the intravitreal class is the one with the higher mean $\mathbf{M = 0.21; SD = 0.082.}$

In table 5, we observe the existence of statistically significant differences regarding the method of administration between Intravenous and Intravitreal classes ($\mathbf{p < 0.05}$), and between subcutaneous and intravitreal classes ($\mathbf{p < 0.05}$).

Table 5 – Multiple Comparisons (Pairwise Method), of ADAs frequency, relative to the Method of Administration.

Sample 1-Sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig.[a]
Intravenous-Subcutaneous	5.117	6.787	0.754	0.451	1.000
Intravenous-Intravitreal	-49.747	14.947	-3.328	0.001	**0.003**
Subcutaneous-Intravitreal	-44.631	15.035	-2.968	0.003	**0.009**

a. The significance values were adjusted by Bonferroni correction for several tests. This method was used to control the family-wise error rate.

Still, in table 4, we can observe the statistically significant differences in the nAbs Index, relative to the Method of Administration($\mathbf{H(45) = 7.073; p < 0.05}$), and in this case the class with a higher median value is the Intravenous class $\mathbf{M = 0.02; SD = 0.056}$.

These differences (table 6) are found between the Intravitreal and Intravenous classes ($\mathbf{p < 0.05}$) and between the Intravitreal and Subcutaneous classes ($\mathbf{p < 0.05}$).

Table 6 - Multiple comparisons (Pairwise Method), of the nAbs frequency related with the Method of Administration.

Sample 1-Sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig.[a]
Intravitreal-Intravenous	35.385	14.163	2.498	0.012	**0.037**
Intravitreal-Subcutaneous	37.672	14.246	2.644	0.008	**0.025**
Intravenous-Subcutaneous	2.288	6.431	0.356	0.722	1.000

a. The significance values were adjusted by Bonferroni correction for several tests. This method was used to control the family-wise error rate.

Biological medicinal products with regulatory and enzymatic activity

Hypothesis 3 – Differences between the frequency of ADAs and nAbs related to the type of product

Dependent Variables: ADAs and nAbs frequency;

Independent Variables: Type of Product (Enzyme, Hormone, Insulin, IFN, GM-CSF, Factor VIII, Human Factor VIII, Human Factor IX, Human Cell and Tissue Product)

To evaluate if the variable "Type of product" conditions the frequency of ADAs and nAbs, and since the assumption of normality for more than two independent samples has not been met, the Non- Parametric method of Kruskal-Wallis was used.

Table 7- Non-Parametric method Kruskal-Wallis (H) for independent samples, from the frequencies of ADAs and nAbs, relative with the type of product.

Type of Product	ADA	nAb
	M ± SD	M ± SD
Enzyme	0.20 ± 0.252	0.07 ± 0.138
Hormone	0.20 ± 0.012	0.00 ± 0.000
Insulin	0.00 ± 0.000	0.00 ± 0.000
IFN	0.01 ± 0.014	0.00 ± 0.003
GM-CSF	0.00 ± 0.000	0.00 ± 0.000
Factor VIII	0.00 ± 0.003	0.00 ± 0.010
Human Factor VIII	0.00 ± 0.000	0.00 ± 0.007
Human Factor IX	0.00 ± 0.000	0.00 ± 0.000
Human Cell and Tissue Product	0.00 ± 0.000	0.00 ± 0.000
H	43.950	16.972
p	**0.000**	0.075

Table 7 shows statistically significant differences in the index of ADAs, regarding the type of product ($\mathbf{H(33) = 43.950; p < 0.05}$), being the classes of enzyme and hormones the

ones that present higher mean values $\mathbf{M = 0.20; SD = 0.252}$ and $\mathbf{M = 0.20; SD = 0.012}$ respectively.

The multiple comparisons test between the different types of products, as contemplated in Table 8, showed that these differences are mainly found between the classes insulin and enzyme ($\mathbf{p < 0.05}$), GM-CSF and enzyme ($\mathbf{p < 0.05}$), human factor VIII and enzyme ($\mathbf{p < 0.05}$), and between human factor IX and enzyme ($\mathbf{p < 0.05}$). This Pairwise method aims to detect any significant different between two groups.

Table 8 - Multiple Comparisons (Pairwise Method) of the ADAs frequency and the type of product

Sample 1-Sample 2	Test statistic	Std. error	Std. test statistic	Sig.	Adj. Sig.[a]
Insulin-Enzyme	33.060	9.532	3.468	0.001	**0.029**
GM-CSF-Enzyme	33.060	7.280	4.541	0.000	**0.000**
Human Factor VIII-Enzyme	33.060	8.845	3.738	0.000	**0.010**
Human factor IX-Enzyme	33.060	7.903	4.183	0.000	**0.002**

a. The significance values were adjusted by Bonferroni correction for several tests. This method was used to control the family-wise error rate.

For the variable "Frequency of nAbs", no statistically significant differences were identified.

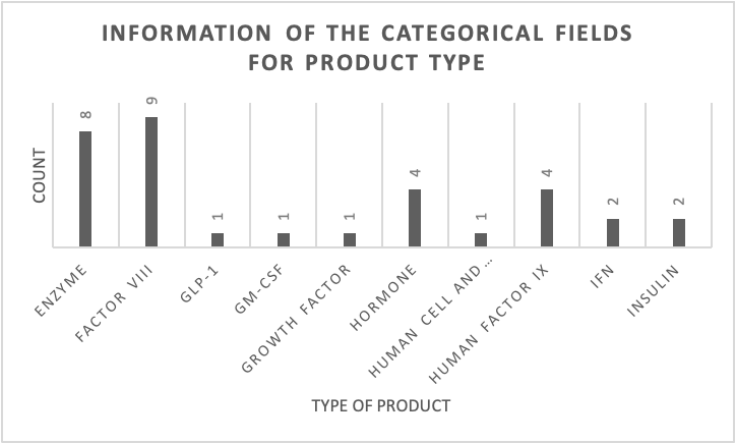

Figure 4 – Categorical fields for the product type (n=33).

Hypothesis 4 – Differences between the frequency of ADAs and nAbs regarding the method of administration

Dependent Variable: ADAs and nAbs frequency

Independent Variable: Method of Administration (Subcutaneous, Intravenous and Intravitreal)

To analyze if the Method of Administration (Subcutaneous, Intravenous and Intravitreal) impacts the frequency of ADAs and nAbs, and since the assumption of normality for the three independent samples, the Non-Parametric method of Kruskal-Wallis (H) was used.

Table 9 - Non-Parametric method of Kruskal-Wallis (H) for independent samples of the frequencies ADA and nAb regarding the method of administration.

Administration Method	ADA	nAb
	M ± SD	M ± SD
Subcutaneous	0.10 ± 0.265	0.05 ± 0.151
Intravenous	0.09 ± 0.136	0.02 ± 0.039
Intraarticular	0.00 ± 0.000	0.00 ±0 .000
Intralesional	0.00 ± .000	0.00 ± 0.000
Intramuscular Intravenous	0.00 ± 0.000	0.00 ± 0.000
H	43.950	16.972
P	**0.000**	0.075

Table 9 shows that only ADAs had statistically significant differences related with the method of administration ($\mathbf{H(33) = 43.950; p < 0.05}$), being the class of subcutaneous administration the one with higher mean values ($\mathbf{M = 0.10; SD = 0.265}$).

When running a Pairwise Method, we find in table 10 that the differences are more striking between the two groups intralesional and intravenous ($\mathbf{p < 0.05}$).

Table 10 - Multiple Comparisons (Pairwise Method) of the frequency of ADAs, regarding the method of administration.

Sample 1-Sample 2	Test statistic	Std. error	Std. Test statistic	Sig.	Adj. Sig.[a]
Intralesional-Intravenous	21.403	6.955	3.077	0.002	0.044

a. The significance values were adjusted by Bonferroni correction for several tests. This method was used to control the family-wise error rate.

Considering the variable frequency of nAbs, no statistically significant differences were detected regarding the Method of Administration.

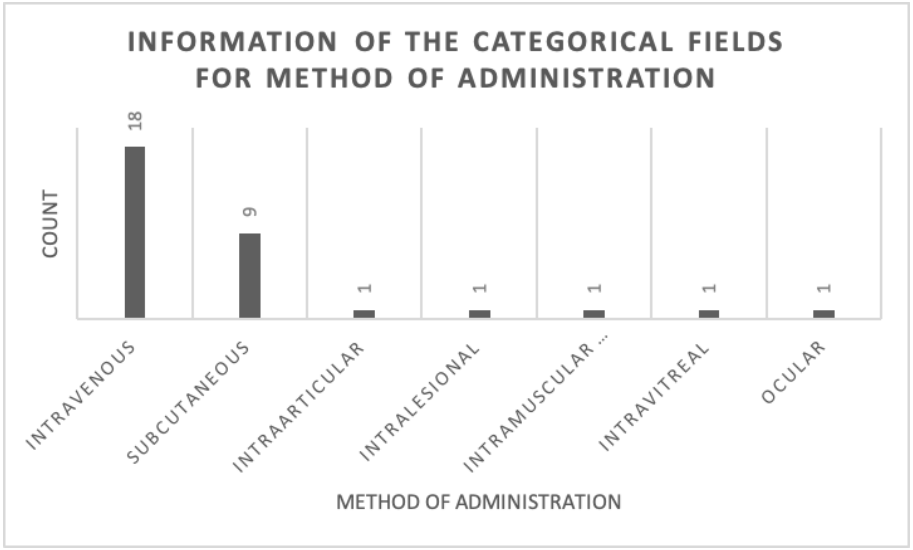

Figure 5 - Categorical fields for the method of administration.

All studies above show that both the method of administration and product type influence the frequency of ADAs and nAbs. The class of medicinal products with targeting activity only contemplated mAbs and FcFPs, since only these two product types had enough data to be studied or were approved in the last 10 years. The group with enzymatic and regulatory activity shows a wider variety of product types.

Monoclonal antibodies show a higher frequency of ADAs and nAbs when comparing with the FcFPs. The method of administration who has shown a statistically significant

difference is the intravitreal administration, in which around 25% of patients developed ADAs. Following intravitreal administration, the biological medicines administered intravenously are the ones to show higher frequencies of ADAs and nAbs.

For the medicinal products within the group with regulatory and enzymatic activity, hormones and enzymes showed higher frequencies of ADAs when compared to other product types of the same group. However, no statistically significant differences between these medicinal products were found regarding nAbs. The method of administration which seems to show higher frequencies of ADAs and nAbs is the subcutaneous administration.

Although this data was collected from the public assessment reports summaries in which the approval of these medicines relied on, many problems can be associated with the frequencies presented above. The type of immunoassays used can highly impact the results obtained, for example, the group with regulatory and enzymatic activity did not show statistically significant results regarding nAbs, however, the literature advocates that these type of products (hormones, enzymes, etc.) show greater values of nAbs when compared to ADAs. Additionally, the number of patients enrolled in the clinical trials was small. A very important aspect of this evaluation relies on the frequencies of ADA and nAbs, which were not presented in the same manner and were not collected at the same time (day, week of the study). The time of collection of samples is extremely important when it comes to the detection of ADAs or nAbs, since it can influence its detection levels and can play an important role in the frequencies reported. The differences in the duration of the study can also interfere with the results.

Immunogenic adverse reactions

As already mentioned, all immunogenic adverse reactions are triggered by the development of ADAs and nAbs against the therapeutic proteins. For this reason, and after the study of their onset regarding the method of administration and the type of product, all reported immunogenic adverse events related with the MedDRA SOC "Immune system disorders" were retrieved from the SmPCs.

Frequency of immunogenic adverse reactions reported for biological medicines with targeting activity

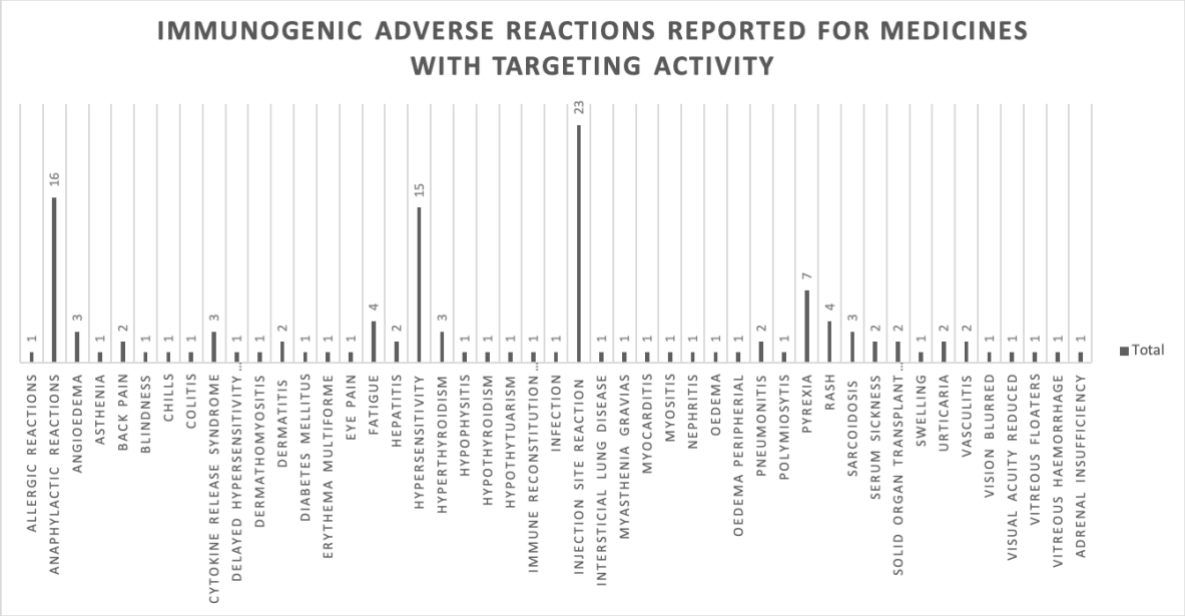

Figure 6 - Frequency of the immunogenic adverse events reported for the group of biological medicines with targeting activity.

In total, 47 different adverse events were reported regarding all products with targeting activity. The most adverse reactions reported are injection site reactions which include pain, induration, erythema, pruritus and rash, followed by anaphylactic reactions, hypersensitivity, pyrexia, rash, fatigue, angioedema, cytokine release syndrome, hyperthyroidism and sarcoidosis.

The following figures shows the frequency of these immunogenic adverse reactions according to the methods of administration of the medicinal product.

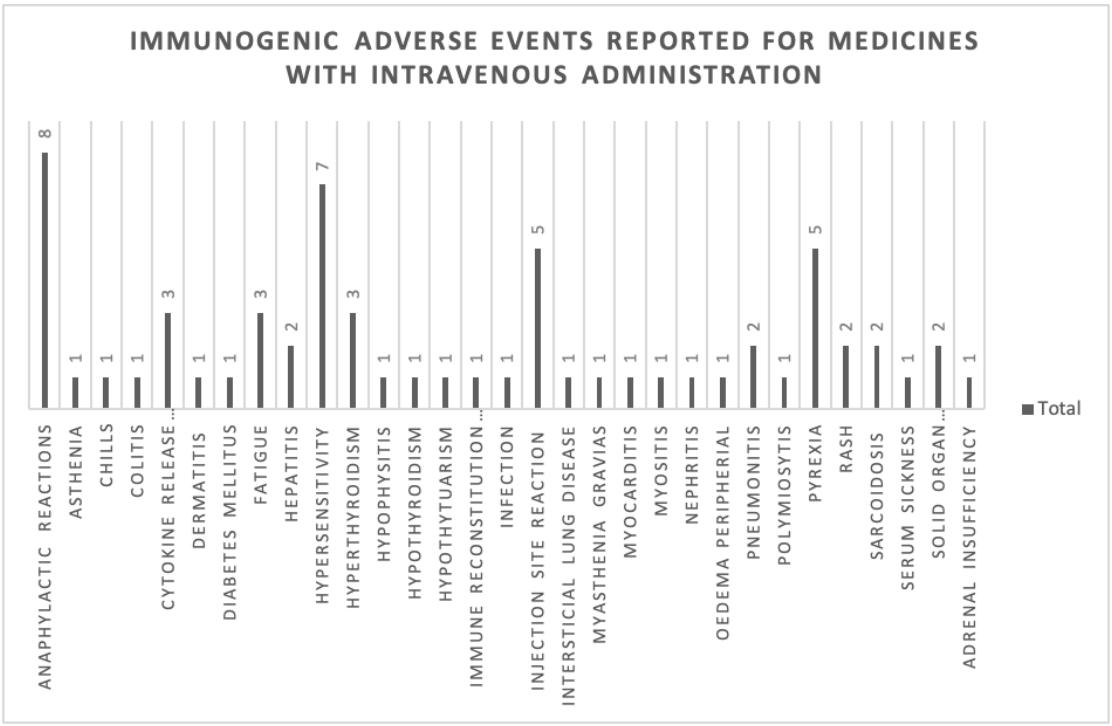

Figure 7 - Frequency of immunogenic adverse reactions reported for medicines with intravenous administration (medicines with targeting activity).

Figure 7 shows that the most reported adverse events regarding the intravenous method of administration are anaphylactic reactions, hypersensitivity, injection site reactions and pyrexia.

For therapeutic proteins with subcutaneous administration, the frequency of anaphylactic reactions and hypersensitivity is the same as for the medicines with intravenous administration as showed in figure 8 below. However, the subcutaneous administration seems to develop more injection site reactions.

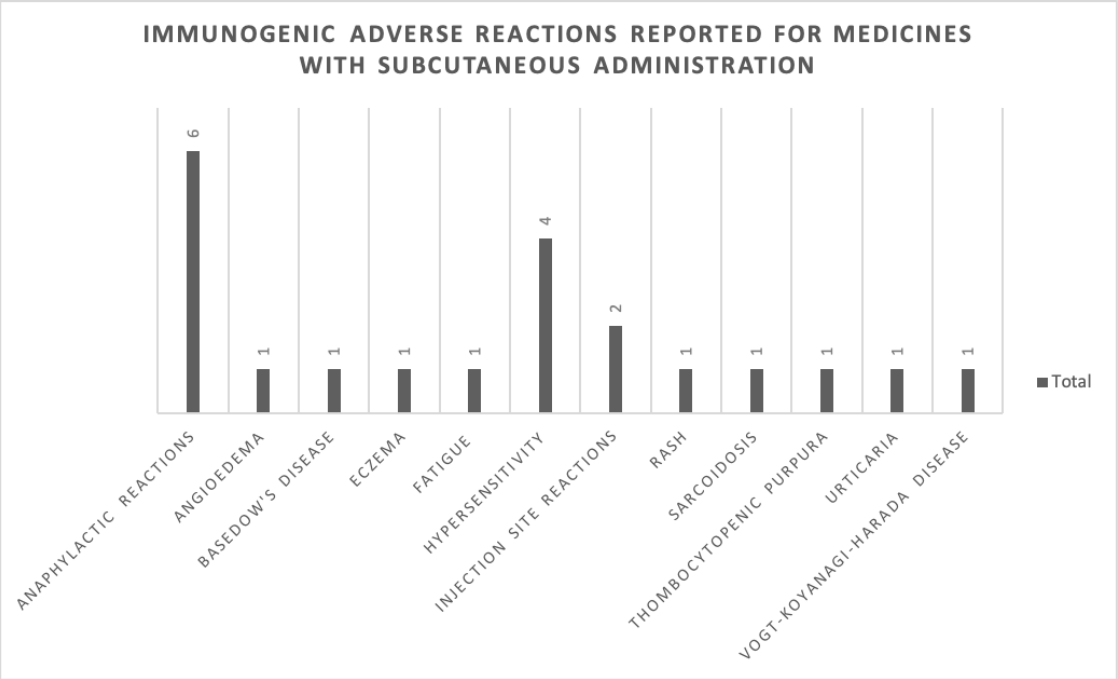

Figure 8 - Frequency of immunogenic adverse reactions reported for medicines with subcutaneous administration (medicines with targeting activity).

For the medicinal products with intravitreal administration the most reported adverse events were blindness, eye pain, hypersensitivity, vision blurred, vision acuity reduced, vitreous floaters and vitreous haemorrhage. These events were not reported for the medicinal products with other administration methods, which indicates that these reactions are only linked to this type of administration.

Table 11 reflects the frequency of these adverse reactions according to the detection of ADAs and/or nAbs.

Table 10 - Frequency of the adverse reactions related with ADAs and nAbs for the group of biological medicines with targeting activity.

Adverse reactions	ADA		nAb	
	n	%	n	%
Anaphylactic Reactions	14	36,84	10	45,45
Hypersensitivity	13	34,21	6	27,27
Injection Site Reactions	22	57,9	16	72,72
Pyrexia	4	10,53	1	4,54
Rash	4	10,53	2	9,09
Fatigue	3	7,9	0	0
Angioedema	3	7,9	3	13,63
Cytokine release syndrome	1	2,63	0	0
Hyperthyroidism	3	7,9	2	9,09
Sarcoidosis	3	7,9	2	9,09
……	…	…	…	…
Total	38	100,0	22	100,0

The active substance durvalumab is by far the one with highest number of adverse events related to the SOC Immune System Disorders, with 19 possible reactions reported. On the other hand, the active substances brentuximab vedotin and bezlotoxumab do not have any reports for adverse reactions related with this SOC. Other active substances such as erenumab, brolucizumab and etanercept have a mean of 7 different adverse reactions associated with immunogenic responses. Many other adverse reactions were also associated with this type of therapeutic proteins, but do not have such a high frequency when compared with the ones in the table above.

Frequency of immunogenic adverse reactions reported for biological medicines with enzymatic or regulatory activity

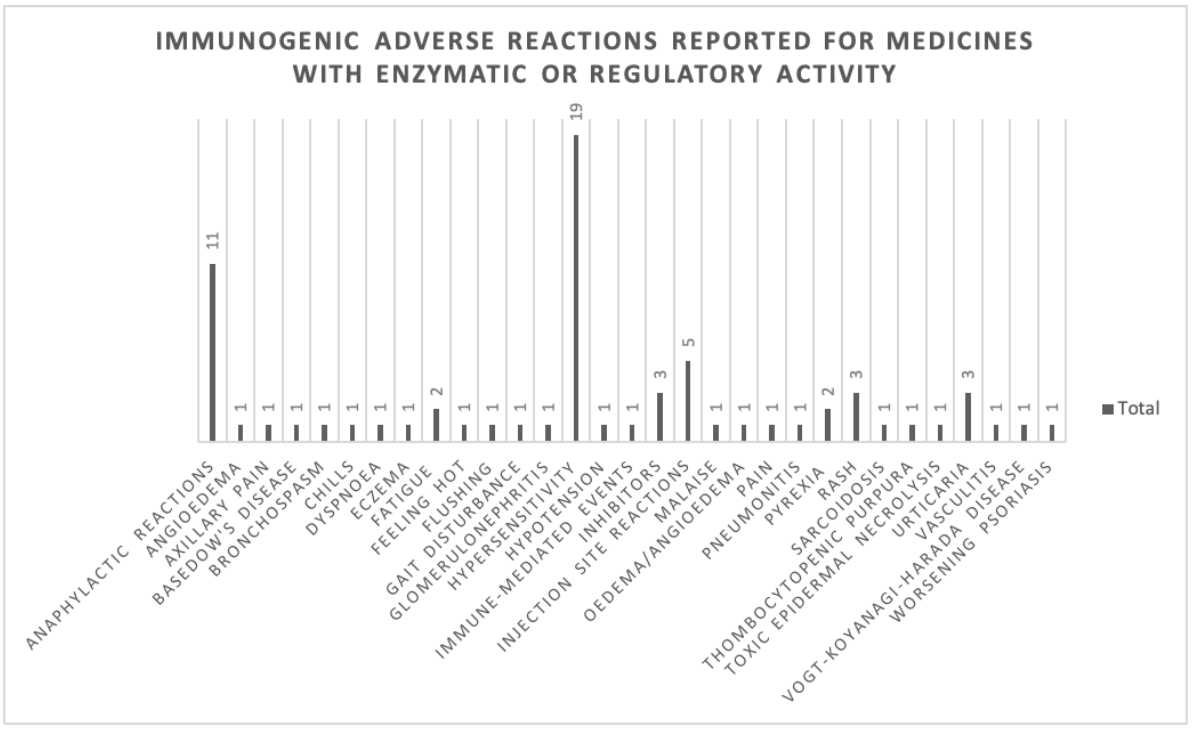

Figure 9 - Frequency of the adverse events reported for the group of biological medicines with enzymatic or regulatory activity.

In total, 31 different adverse events were reported regarding all products with enzymatic or regulatory activity. The most adverse reactions reported are hypersensitivity, anaphylactic reactions, injection site reactions, inhibitors, rash and urticaria.

The following figures show the frequency of these adverse reactions according to the methods of administration of the medicinal product. This group of biological medicines presents more methods of administration comparing to the group of medicines with targeting activity. However, only the subcutaneous and intravenous methods will be further discussed since these have a more significant number of reports. In contrast to our previous study, regarding the medicinal products with targeting activity, this group has no reported immunogenic adverse events for medicines administered intravitreally.

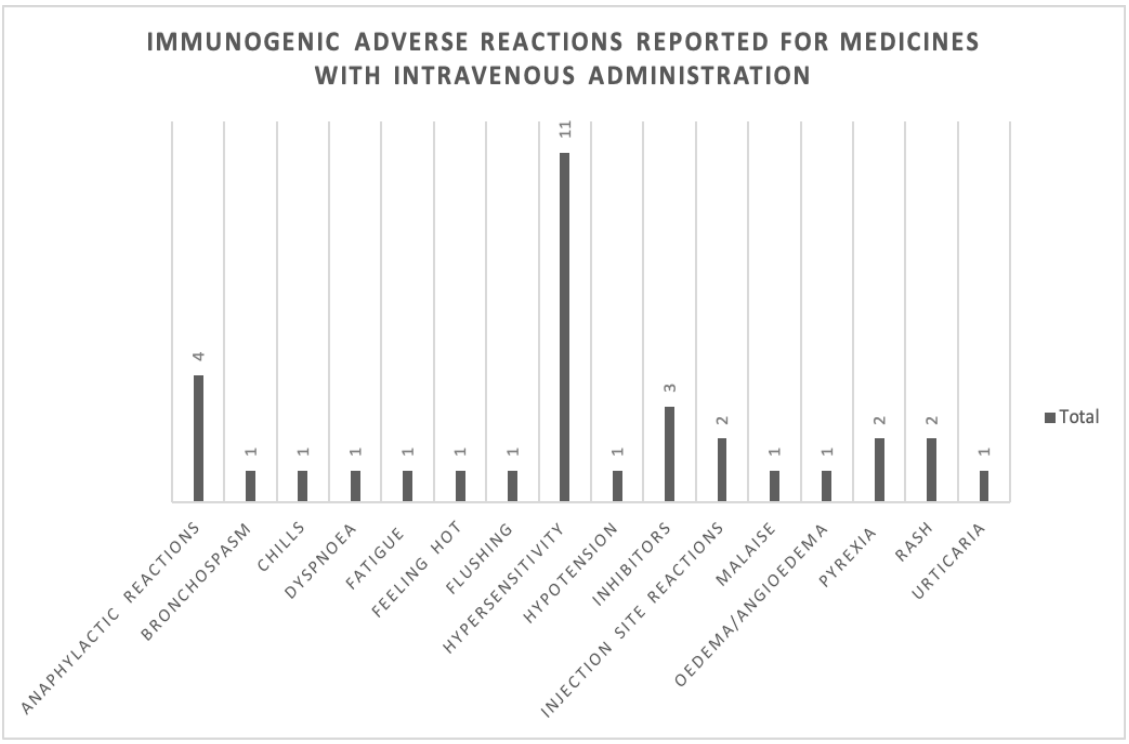

Figure 10 - Frequency of immunogenic adverse reactions reported for medicines with intravenous administration (medicines with enzymatic or regulatory activity).

Figure 10 shows that for medicines with intravenous administration the most frequent adverse reactions are hypersensitivity, anaphylactic reactions and inhibitors.

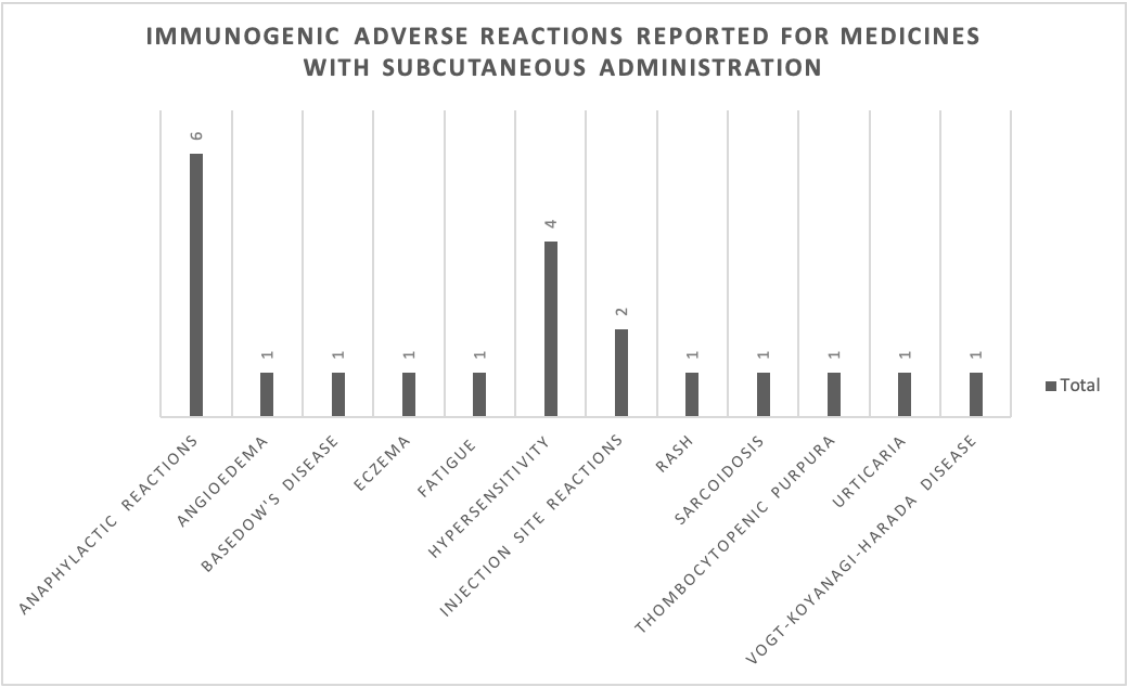

Figure 11 - Frequency of immunogenic adverse reactions reported for medicines with subcutaneous administration (medicines with enzymatic or regulatory activity).

According with figure 11, the subcutaneous method of administration seems to be related with anaphylactic, hypersensitivity and injection site reactions. Although hypersensitivity reactions are also linked to this type of administration, it can be inferred by its frequency that it is less related when compared to intravenous administration.

Table 12 reflects the frequency of these adverse reactions according to the detection of ADAs and/or nAbs.

Table 11- Frequency of adverse reactions related to ADA and nAb for the group of biological medicines with regulatory and enzymatic activity.

Adverse Reactions	ADA		nAb	
	n	%	n	%
Anaphylactic Reactions	7	58,33	3	60
Hypersensitivity	4	33,33	4	80
Injection site reactions	3	25,00	1	20
Rash	1	4,54	1	20
Urticaria	1	4,54	1	20
……	…	…	…	…
Total	12	100,0	5	100,0

This group of medicinal products has a lower number of reported adverse reactions contemplating a total of 31 adverse reactions, when compared with the biological medicines with targeting activity. The active substances ropeginterferon alfa-2b, asparaginase and velmanase alfa showed a median number of approximately 6 adverse reactions reported. The adverse reactions of fatigue and pyrexia seem to have only been reported for patients for which only ADAs were detected.

These results can be indicative; however, it is difficult to predict the origin and causes of the onset of the adverse reactions rash and urticaria since they were only detected for one of the biological medicines studied. Although with no statistical significance, anaphylactic reactions

and hypersensitivity seem to be related to higher detection of ADAs. It is important to better evaluate the immunogenicity for these types of medicines, since EMA believes that ADAs and nAbs should be detected, which is not the case in this group of biological medicines, since most immunogenicity summaries indicate that although analysed neither ADA nor nAb have been identified.

5. Conclusion

Biological medicines play a crucial role when it comes to treating diseases with no effective treatment and filled a huge gap of the pharmaceutical industry at the time of their discovery, the reason why their development was and continues to be important.

Their development has also brought major challenges at a regulatory, clinical and financial level and for this reason, patients, prescribers and National Health Systems had to adapt to a new reality which included new therapeutic regimens, an adaptation of pharmacovigilance systems and increased efforts regarding funding. Considering the diversity of therapeutic proteins, setting solid regulatory principles constitute an important role in ensuring their efficacy and safety.

Both EMA and FDA are responsible for the regulatory evaluation of these medicines in European Union/European Economic Area and the United States of America, respectively. Although initially, the regulatory principles of these agencies were different, their convergence over time has been noted. Despite some methodology differences regarding the regulation of biological medicines, nowadays the principles of both agencies (FDA and EMA) have more in common than otherwise, which has allowed for greater acceptance and conformity regarding the methods to be considered during these evaluations. This allowed a greater conformity between the biological medicines approved in the United States and in the European Union.

Immunogenic adverse reactions to therapeutic proteins are still one of the greatest challenges for regulators, and some of the reasons for their occurrence are still unknown. Although their occurrence is not completely understood many factors like the method of administration, type of product and dosage can be related with their onset, and to determine the immunogenicity of a biological product ADAs and nAbs should be evaluated. To execute this type of study correctly, both agencies request the submission of an immunogenicity study together with the MAA, and this should be based on a risk approach and most importantly

specific to each product, and carried out in a target population who has not been previously exposed to the medicine in study. FDA and EMA have issued different guidances concerning the assessment of immunogenicity and specify what is considered crucial to be evaluated for each one of them. The guidance for industry issued by FDA contains more information than the one issued by EMA, and also provides more information for the diagnosis and reporting of acute delayed immune responses, important for pharmacovigilance monitorization. On the other hand, EMA requests a risk management plan and allows MAAs more freedom when developing its own study. Non-clinical studies are not considered to be sufficiently predictive of the immunogenicity of medicines, however, play a considerable role in predicting the test specificity which is very important for accurate detection of ADAs and nAbs during clinical trials. FDA has defined a sensitivity up to 100 nanograms/ml, while EMA has not defined a sensitivity value for the tests undertaken.

Throughout the last 10 years, around 78 biological medicines have been approved in the EU/EEA and the data contemplating the immunogenicity of all of these medicines were collected and evaluated from the European Public Assessment Reports (EPARs).

For the biologicals with targeting activity, higher levels of ADAs $(U(5) = 154.000; p < 0.05)$ and nAbs $(U(45) = 189.000; p < 0.05)$ were detected for mAbs. Intravitreal administration had a significant difference for ADAs $(H(45) = 11.078; p < 0.05)$, and intravenous administration for nAbs $(H(45) = 7.073; p < 0.05)$. As regards to the group with enzymatic or regulatory activity the, enzymes and hormones showed significant differences for ADAs as well as the subcutaneous administration - $(H(33) = 43.950; p < 0.05)$. No significant differences were identified for nAbs within this group. Although the literature established a higher relation between the ADA levels and the medicines in the group with targeting activity and more nAbs levels in the group with enzymatic or regulatory activity, when comparing the median values of ADAs and nAbs reported in the summaries of immunogencity,

products such as hormones and enzymes do have indeed higher medians of nAbs, however these products show also higher median of ADAs when comparing to medicinal products such as mAbs.

Because of ADA and nAb development, immunogenic reactions occur for patients treated with biological medicines, and for this reason, it is extremely important to study their occurrence. The most-reported immunogenic adverse reactions for the medicinal products for the group with targeting activity were injection site reactions, anaphylactic, hypersensitivity and pyrexia reactions. Injection site reactions and anaphylactic reactions represent a total of approximately 37% and 58% of the total of reactions reported for medicines with ADAs detection. For the medicines with nAb detection both of these reactions represent 73% and 58%, respectively. The most reported reaction for medicines with intravenous administration were anaphylactic reactions, hypersensitivity and injection site reactions. For medicines administered subcutaneously the most reported adverse reaction was anaphylaxis.

For the therapeutic proteins on the group with enzymatic or regulatory activity, from a total of 31 possible adverse events, the most reported were anaphylaxis and hypersensitivity, rash and urticaria. For the medicines in which ADAs were detected the anaphylactic reactions and hypersensitivity represent 59% and 33%, respectively. For the same adverse reactions but with medicines with nAb detection these correspond to 60% and 80%, respectively.

Many more immunogenic adverse reactions were reported for this SOC, but in most cases, in only one medicinal product, and for this reason it was difficult to predict the reason for their onset.

Thus, pharmacovigilance activities are crucial, to predict the possible reasons for their onset during the post-authorisation period. Although many advances have been conquered, there is a necessity to further relate the occurrence of adverse reactions with possible onset of ADAs and/or nAbs as well as the identification of the product name in a pharmacovigilance

context. A total of 208 304 adverse reactions have been reported for these therapeutic proteins but only a few were related with the SOC “Immune adverse events”. Of this total, 8% corresponds to medicines with enzymatic or regulatory activity and approximately 92% for medicines with targeting activity.However, the results obtained in this study suggest that the data presented to regulatory authorities at the time of marketing authorization was not accurate, since the literature indicates that the group with enzymatic or regulatory activity, such as hormones and enzymes, trigger more nAbs than ADAs, which is not evidenced by the data presented. Additionally, the therapeutic proteins belonging to the group with targeting activity, such as mAbs, should trigger more ADAs, which is also not the case.

For all the reasons mentioned above, it is extremely necessary to adjust the methodology used in every non-clinical and clinical phases of the development, to detect in a more precise manner the ADAs and nAbs onset. The information presented in the summary of immunogenicity in the EPARs is not submitted in the same manner for all medicines, and some of them have not performed immunogenicity tests. This information highlights the importance of performing ADAs and nAbs detection in the different phases of the study, with identification of pre-dose sampling, follow-up period and differentiation between the placebo and active treatment groups with the most specific test possible. It seems that medicinal products approved more recently, such as durvalumab, present more detailed and accurate information, the reason why some older EPARs and immunogenicity tests should be reviewed accordingly. Also, the role of the nAbs should be assessed since their impact and importance does not seem to be well established, considering that the risk-benefit approach is based on the impact of ADAs in pharmacokinetics.

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