Ana Leonor Pereira Bruçó

Orphan Drugs: Development Process and Clinical Use

Dissertação apresentada à Faculdade de Farmácia da Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia Farmacêutica realizada sob a orientação científica do Professor Doutor Sérgio Paulo Magalhães Simões e do Professor Doutor Francisco Luís Pimentel

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"(…)
E os passos que deres,
Nesse caminho duro
Do futuro
Dá-os em liberdade.
Enquanto não alcances
Não descanses.
De nenhum fruto queiras só metade.
(…)"

Miguel Torga *in* Diários vol. XIII-XVI

Ao meu Pai,
por me ter ensinado que sempre que o homem sonha,
o mundo pula e avança como uma bola colorida entre as mãos de uma criança.

À minha Mãe,
por me ter ensinado que como uma gaivota que voava e voava,
com asas de vento e coração de mar, também nós somos livres de voar.

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Às minhas colegas de casa por tornarem este percurso mais alegre.

Sem vocês não seria possível chegar até aqui. A todos MUITO OBRIGADO!

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LIST OF ACRONYMS

AAV	Adeno-Associated Virus
ACVR1	Activin A Receptor type 1
ADA-SCID	Adenosine Deaminase Deficiency - Severe Combined Immunodeficiency
ALK	Anaplastic Lymphoma Kinase
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS Functional Rating Scale
AMED	Agency for Medical Research and Development
AO	Antisense Oligonucleotide
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CHMP	Committee for Medicinal Products for Human Use
COMP	Committee for Orphan Medicinal Products
DMD	Duchenne Muscular Dystrophy
DNA	Deoxyribonucleic Acid
EC	European Commission
EMA	European Medicines Agency
ERT	Enzyme Replacement Therapy
ESMO	European Society for Medical Oncology
EU	European Union
FDA	Food and Drug Administration
FP	Framework Programmes
GAA	Glucosidase Alfa Acid
GBA	β-Glucocerebrosidase
GUSB	β-Glucuronidase
iPSC	induced Pluripotent Stem Cells
LPL	Lipoprotein Lipase
LSD	Lysosomal Storage Disorder
MA	Marketing Authorisation
MHLW	Minister of Health, Labour and Welfare
MJD	Machado-Joseph Disease

MPS	Mucopolysaccharidosis
mRNA	Messenger RNA
miRNA	Micro-RNA
NIBIO	National Institute of Biomedical Innovation
NIH	National Institutes of Health
NORD	National Organization for Rare Diseases
NSCLC	Non-small Cancer Lung Cancer
ODA	Orphan Drug Act
OOPD	Office of Orphan Products Development
OPCTGP	Orphan Products Clinical Trials Grants Programme
PAFSC	Pharmaceutical Affairs and Food Sanitation Council
PD-1	Programmed Death 1
PINK1	PTEN-Induced Putative Kinase 1
PMDA	Pharmaceutical Medical Devices Agency
RCT	Randomized Controlled Trial
RNA	Ribonucleic Acid
SLC	Solute Carrier
SMA	Spinal Muscular Atrophy
SMN	Survival Motor Neuron
SNCA	α-Synuclein
SOD	Superoxide Dismutase
SRT	Substrate Reduction Therapy
TGF-β	Transforming Growth Factor-β
USA	United States of America
WHO	World Health Organization

ABSTRACT

Rare diseases have become a topic of substantial interest in recent years. These disorders are usually severe, have a genetic origin and their symptoms are generally expressed at a young age. In most cases, there is no effective treatment for patients with these conditions. Therefore, the search for new therapeutic solutions for these patients has been increasing. Medicinal products used to prevent, diagnostic or treat rare diseases are called orphan drugs. Regulatory authorities, pharmaceutical companies, academic researchers and international organizations have contributed with several efforts to facilitate the development process for these drugs.

Despite those accomplishments, clinical research is particularly difficult in rare diseases, as researchers are faced with evident obstacles, such as low disease prevalence, small and heterogeneous patient populations, clinical trials recruitment difficulties and lack of scientific data on this kind of diseases.

These demands require the development of novel and rigorous clinical study designs and analyses to assess treatment efficacy properly in small populations. Progresses in genetics and biotechnology can provide important tools to improve clinical trial evaluation methods. The preclinical and clinical development process, regulatory framework and market for orphan drugs will be discussed in this dissertation, as well as some therapeutic approaches for rare diseases. Relevant considerations about some rare diseases and possible future scenarios will be also given.

Keywords: rare diseases, orphan drugs, clinical trials, clinical study designs.

RESUMO

As doenças raras tornaram-se um tópico de grande interesse nos últimos anos. Estas doenças são geralmente graves, têm origem genética e os seus sintomas manifestam-se habitualmente numa idade jovem. Na maioria dos casos, não existe um tratamento eficaz para os doentes com estas condições. Por isso, a procura por novas soluções terapêuticas para estes doentes tem vindo a aumentar. Os medicamentos utilizados para prevenir, diagnosticar ou tratar doenças raras designam-se medicamentos órfãos. As autoridades regulamentares, as empresas farmacêuticas, os investigadores académicos e as organizações internacionais têm feito vários esforços no sentido de agilizar o processo de desenvolvimento destes medicamentos.

Apesar destes progressos, a investigação clínica em doenças raras continua a ser particularmente difícil, uma vez que os investigadores se deparam com obstáculos evidentes como a baixa prevalência da doença, uma população de doentes pequena e heterogénea, dificuldades no recrutamento dos ensaios clínicos e a falta de conhecimento científico neste tipo de doenças.

Estas exigências requerem o desenvolvimento de ensaios clínicos com desenhos novos e rigorosos e métodos de análise que avaliem adequadamente a eficácia do tratamento em populações pequenas. Os avanços na área da genética e da biotecnologia podem oferecer ferramentas importantes para melhorar os métodos de avaliação dos ensaios clínicos. O processo de desenvolvimento pré-clínico e clínico, o enquadramento regulamentar e o mercado dos medicamentos órfãos será discutido nesta dissertação, assim como algumas abordagens terapêuticas para doenças raras. Considerações relevantes sobre algumas doenças raras e possíveis cenários futuros também serão abordados.

Palavras-chave: doenças raras, medicamentos órfãos, ensaios clínicos, desenhos de ensaios clínicos.

1. INTRODUCTION

1.1. RARE DISEASES AND ORPHAN DRUGS

Rare or orphan diseases are defined by their prevalence differently in each country. For example: in the United States of America (USA), a disease is considered as a rare disease when it affects fewer than 200 000 individuals [1]; in Japan the number changes to 50 000 people [2]; in the European Union (EU) the definition comprises conditions that affect no more than 5 out of 10 000 individuals [3]. According to the World Health Organization (WHO), a rare or orphan disease is a condition that affects between 0.65 and 1 in 1000 people [4]. Besides disease prevalence, severity of the disease and therapeutic options available are also taken into account when defining a disease as rare [5] [6]. Furthermore, these diseases are often called rare or orphan because the cost of the investment in new therapeutic approaches for small populations is not affordable [7].

Despite global differences in the definition of rare disease, there is a worldwide effort to create a homogeneous drug development process that can increase the approval rate of new therapies for these diseases. Moreover, some entities, such as the National Institutes of Health (NIH) in the USA, do not take into account the number of patients affected when assessing the funding of a research project, which encourages rare disease research [8].

Rare diseases affect around 400 million persons around the world and there are more than 7 000 rare diseases identified worldwide [9] [10]. Although all rare diseases put together have a significant impact, as they are estimated to affects 6-8% of the world population, relatively few have therapeutic options available [11]. Thus, the investment in research is crucial for developing accurate diagnostic methods and treatments. The discovery and development of drugs for rare diseases, so-called orphan drugs, has grown substantially in the past few years due to relevant advances, for example in the field of genetics, which has given us a better understanding of the pathophysiology of these diseases and, consequently, facilitating their diagnosis.

About 80% of rare diseases have a genetic origin, in which 50% of the affected population are children and 30% die before the age of 5 [8] [12]. Because of this, searching for effective solutions for these diseases represents also an opportunity for emerging therapeutic approaches like biologics and gene therapy [13].

It is important to mention that orphan drug designation is not restricted to drugs for the treatment of rare diseases. It includes also therapies for pathologically or genetically

defined distinct sub-groups of common diseases that meet the definition of a rare disease. For instance, treatments for sub-types of Parkinson disease, with a mutation in certain genes such as SNCA and PINK1 fall under the designation of orphan drugs [6] [14]. The same happens with oncologic drugs like niraparib that was approved as an orphan drug for patients with recurrent epithelial ovarian cancer [6] [15]. Another example is redaporfin, a molecule developed to be used in a photodynamic therapy for head and neck cancer that has orphan drug designation for biliary tract cancer/cholangiocarcinoma [16].

Therefore, orphan drug designation is consistent with the principle of personalized medicine, which highlights the need for taking individual variability into account. The orphan drug clinical development process can be used as an example of a personalized experience between the patient and clinical researcher to find a treatment for a specific rare condition. By learning through successful rare cases, advances in precision medicine can be achieved [17] [18].

Clinical researchers and patients play an essential role in the success of rare disease research. The clinical research team needs to ensure safety and clinical improvement of the patient in order to have the best possible clinical outcomes. On the other hand, the patient should follow the protocol and be supported by their families and the health care system. Other stakeholders have an important intervention in this context: Health care systems, by managing the cost/benefit impact that rare disease treatments will bring and adapt itself for the benefit of the patients; Politicians, by passing legislation and programmes that promote research in this field [19]; Patient organizations by giving a voice to these patients and to require the most adequate treatment available. Initiatives like the creation of rare diseases database platforms have already been adopted and demonstrate the effort being done to improve patient focused care through a worldwide collaboration. For instance, platforms like Orphanet represent a way to improve the communication between patients, researchers and clinical centers, which facilitate the implementation of future clinical trials according to the patients' needs [19].

2. ORPHAN DRUGS: PRECLINICAL DEVELOPMENT PROCESS

2.1. DRUG DISCOVERY AND DEVELOPMENT

Drug discovery and drug development processes are particularly challenging for orphan drugs. Due to the limited investigation in rare diseases and their poorly understood pathophysiology, it is more difficult to identify targets and pathways. To discover new compounds that demonstrate their biological activity against a target, so-called lead compounds, tests are performed to screen different substances and verify their expected pharmacological effect [20]. One of the possible tests is the high throughput molecular target-based screen, using biochemical or cell-based assays. This kind of screens can be difficult to perform in unknown targets. Therefore, an alternative strategy is to use a phenotypic screening. The phenotype modifications observed in a cell-based assay that use primary disease cells are used to identify a target. This approach improves the chances of success of screening analysis without knowing the target. Furthermore, the advances in molecular techniques as the whole genome sequencing can facilitate the identification of targets [8]. From a general perspective, target selection is still considerate the main challenge in improving the productivity of the drug discovery and development phase in rare diseases [21].

2.2. PRECLINICAL STUDIES

Before testing drugs in humans, *in vitro* and *in vivo* studies are required to predict efficacy, dosing limits, possible toxicological effects and off-target reactions. These preclinical studies also provide pharmacokinetic information that may support drug formulation. Choosing accurate animal models of disease to evaluate drug safety and efficacy could provide valuable information which is expected to have reproducible results in humans [7].

In the last few years some human based models of diseases have been emerging, such as induced Pluripotent Stem Cells (iPSCs), which uses specific skin fibroblast cells from each patient that are representative of the human disease condition. The basic principle of iPSC is reprogramming adult differentiated cells into new pluripotent cells that, in theory, could be differentiated in any type of cell [22]. For instance, one advantage of iPSCs when compared to other disease models is the ability to be an effective model of a patient specific disease, ensuring a good correlation value and reliable data extrapolation to humans [23]. This would help to shorten the gap between basic research and clinical development in rare diseases field [6].

3. ORPHAN DRUGS: CLINICAL DEVELOPMENT PROCESS

3.1. CHALLENGES

In clinical research, the requirements needed for rare diseases are almost the same when compared those for common diseases. Although some grants could be given to cover phases I, II and III of clinical trials, the planning and conducting of clinical development processes for rare diseases still presents many challenges. By definition, rare diseases have limited number of patients, which makes it difficult to achieve statistically significant outcomes by performing a conventional clinical trial. Likewise, the recruitment process is hampered by wide geographic dispersion of patient populations [6].

Apart from this, there are phenotypic varieties of the diseases, shortening even more, the patient population and causing large variations in drug response [6]. Furthermore, the understanding of disease history is usually poor or unknown, which can lead to the definition of unrealistic endpoints and the application of inappropriate clinical studies designs [6].

3.2. CLINICAL TRIAL DESIGNS AND STRATEGIES

To generate new reliable scientific results, evidence must be grounded in consistent clinical data. The most recognized clinical trial design to evaluate safety and efficacy of treatments is the randomized controlled trial (RCT). RCTs evaluate predefined endpoints, with experimental interventions at defined time points, in different groups randomly chosen among the general population [24]. However, this clinical trial design is difficult to implement in rare diseases due to the limited number of patients.

Small sample size implies necessarily multicenter trials. Prevalence of rare diseases is distinct between several areas and they could have a higher incidence in certain regions, such as Machado-Joseph disease (MJD), a rare neurodegenerative disorder for which the most representative epidemiologic cluster is in the Azores [25]. Accessibility and incorporation of new geographical areas improve the outcome of the recruitment process [26]. Therefore, combining results from several studies and patient pools from other research sites can be a solution. Worldwide collaboration between different clinical teams, patient's organizations and authorities is crucial to achieve meaningful clinical research progress [12] [27].

Another strategy to address small sample size is to use statistical trial designs such as the Bayesian approach, which suggests the incorporation of external and subjective information into the estimation of therapeutic results, complementing the restricted

information available from the study itself, without using a large number of patients [12] [28] [29]. The core principle for Bayesian designs is to focus on estimation rather than hypothesis, obtaining data to decrease uncertainty and assuring the next steps in clinical practice. This approach is useful in increasing trial efficiency, particularly in early phase studies and in adaptive designs [29].

Besides the small sample size, clinical trials in rare diseases must consider how heterogeneous the population is. For that, clinical trials designs like enriched enrolment and randomized withdrawal design are proposed. This design comprises of two main steps. In the first step, called enriched enrolment, all subjects are exposed to experimental treatment and only the ones who response positively are chosen to carry on to the next phase. In the second phase, randomized withdrawal, the previously selected population is randomly allocated in the arm of experimental treatment continuation or in the control arm. Data analysis begins at this phase, increasing this design's statistical power, as that sample consists only of subjects with larger than the average clinical response in comparison to the general population. However, ethical concerns may arise with the allocation of participants in the control arm, as in first phase it was proven that they could potentially benefit from the experimental treatment (Fig. 1) [30] [31].

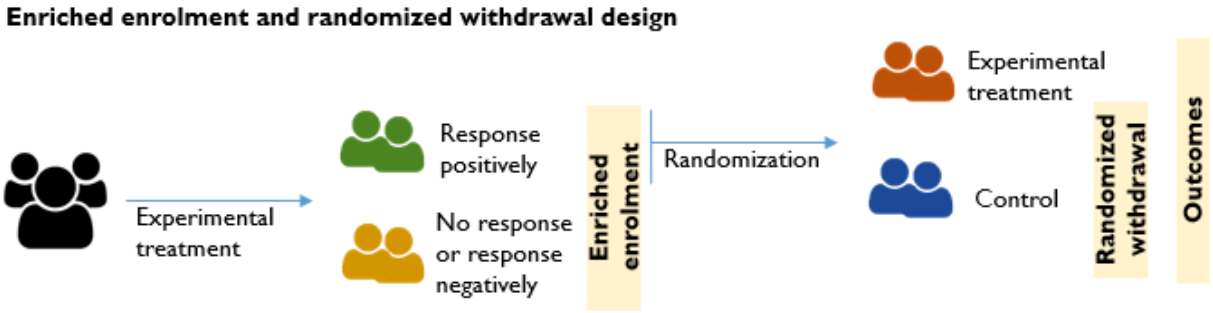

Figure 1: Enriched enrolment and randomized withdrawal trial design. Adapted from source [30].

For small cohorts and unmet diseases, it may be considered unethical to treat patients for whom the control is placebo when an experimental drug could be the only successful therapy available. By using the standard of care therapy as control, instead of placebo, there are clinical trial designs that overcome this limitation. For instance, in cross-over or "N of 1" trials, each patient can be his/her own control if he/she is exposed to both control and treatment [32] [33].

Cross-over trial design enables the participation of selected patients in usually two study treatment arms to compare them sequentially. In a random order, subjects are treated with experimental treatment or with control. After this first period, outcomes are measured, subjects switch from one study arm to the other and the second period begins. Between these two periods, it may be necessary a washout time, which is a period in which the patient refrains from having any experimental intervention until the effects of the previous treatment are no longer relevant [30]. Outcomes are measured again at the end of the study (Fig. 2). This kind of study provides better treatment outcome analysis because it minimizes inter-individual variability and increases statistical power, granting higher acceptability [30].

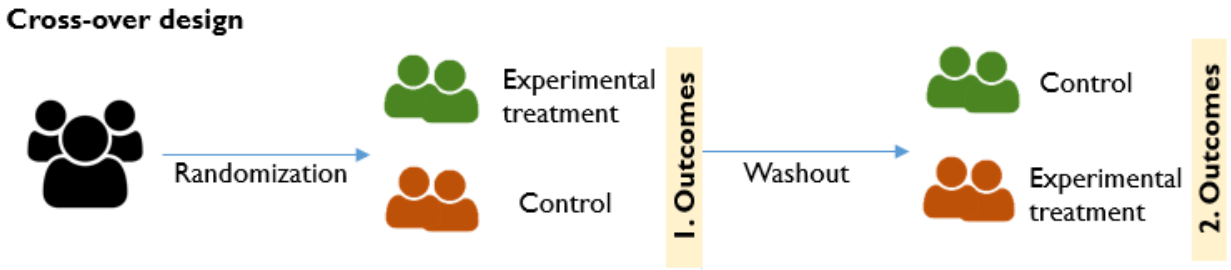

Figure 2: Cross-over trial design. Adapted from source [30].

"N of 1" trial design has the same principles of cross-over design, but it is used to study only a single subject (Fig. 3). The participant receives control or experimental treatment in a randomized sequence over several periods, with the required washout times. Outcomes are measured after each period and analyzed at the end of the study. The combination of these "N-1" trials results may allow researchers to estimate treatment efficacy in the population by using meta-analysis, where several clinical study results are systematically combined to reach a conclusion about the research topic [30] [34] [35].

What is particularly advantageous about this design is its flexibility, as one can extend the study for as many periods as it is required, which facilitates achieving results while having a patient tailored clinical design. Furthermore, in treatments that are expected to have distinct effects across patients it is possible to reach a conclusion about their effectiveness in a single individual, following the direction of personalized medicine principle [30] [36].

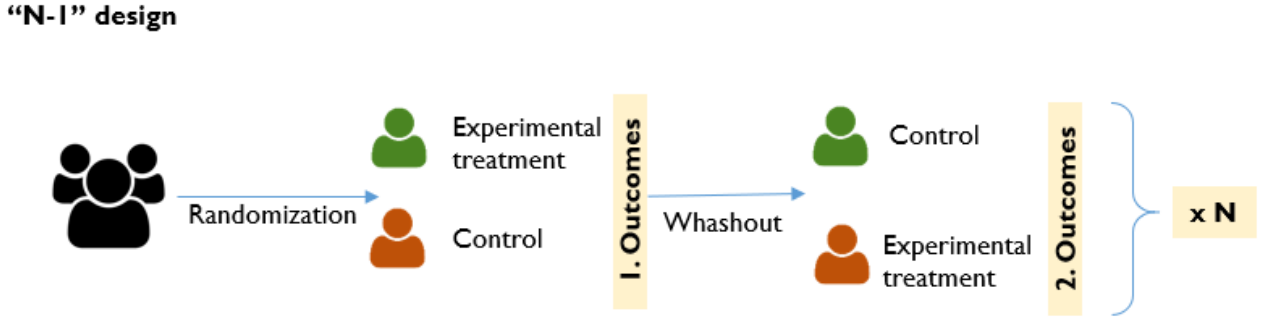

Figure 3: "N-1" trial design. Adapted from source [30].

Nevertheless, for rare diseases, restricted data is better than no data, and case series and reports still hold scientific value which should be put to use. These descriptive studies are a way of recognizing new possible features of patients with rare diseases as they identify unusual cases from clinical practice that may raise new investigational questions [37].

Moreover, in order to overcome the fact of having a limited knowledge about disease history, more emphasis could be put on case-control studies and prospective cohort [38]. Case-control study implies the use of a sample from a cohort of patients that were exposed to certain conditions instead of considering all the information on all patient's cohorts, which restricts the study variables and the ability to provide reliable conclusions and find the condition that caused the disease. The prospective cohort is characterized by the evaluation of a condition/intervention before the disease appears, and define a temporality and causality relation between the intervention and the disease [38].

To conclude, due to the challenges found in the clinical development process for rare diseases, it is crucial to use alternative clinical trial designs and strategies focused on the distinctive characteristics of this kind of diseases.

3.3. ADAPTIVE CLINICAL TRIAL DESIGNS

Clinical trials can be designed using a wide array of methods. Adaptive designs have the versatility to improve the efficiency of rare diseases clinical studies. These designs take part of cumulative data obtained from an ongoing trial and enable the modification of certain trial aspects without decreasing the viability of results. As this is a continuous learning process, it allows for modifications across all trial phases according to the results obtained by performing the procedures specified in the protocol. This is done by a data analysis, usually called interim analysis. Modifications can include sample size adjustment, response adaptive randomization and optimal dose finding [39]. These modifications can be done during the study with protocol amendments.

Particularly interesting for rare diseases are adaptive seamless designs. Instead of providing not-independent or restricted clinical trial phases, they enable a combination of clinical phases that can be applied both in early (phase I/IIa) and in later (phase IIb/III) clinical development stage. Focusing on the later stage, phase IIb and phase III can be performed at the same time, which allows dose and treatment selection as well as sample size adjustment [26]. This design randomly allocates participants in several experimental treatment cohorts and a control cohort, which are evaluated in a preliminary interim analysis. Throughout phase IIb/III, another interim analysis is made to compare experimental cohorts and subject response, to decide which had better therapeutic performance. The cohorts that haven't had the expected results drop out. Continuing in this phase, the selected and control arms are compared in order to obtain the confirmatory outcomes (Fig. 4) [40] [39] [41].

For instance, for a Non-Small Cell Lung Cancer (NSCLC), a phase IIb/III clinical trial was suggested to test an immunotherapy agent. The phase IIb occurred with 3 arms to define the dose level followed by a phase III where the sample size was reassessed, according to the interim analysis results [42].

Also, an example of a phase I/IIa trial was proposed to assess the effect of Coenzyme Q10 in Amyotrophic Lateral Sclerosis (ALS). This study evaluated the decline in ALS Functional Rating Scale score. During its first phase, one of two doses (1800 mg or 2500 mg) was selected, according to the tolerability presented, to transit to the next stage where it is compared to placebo [43].

Using adaptive seamless design, it is possible to shorten the time between clinical phases and making the best use of resources.

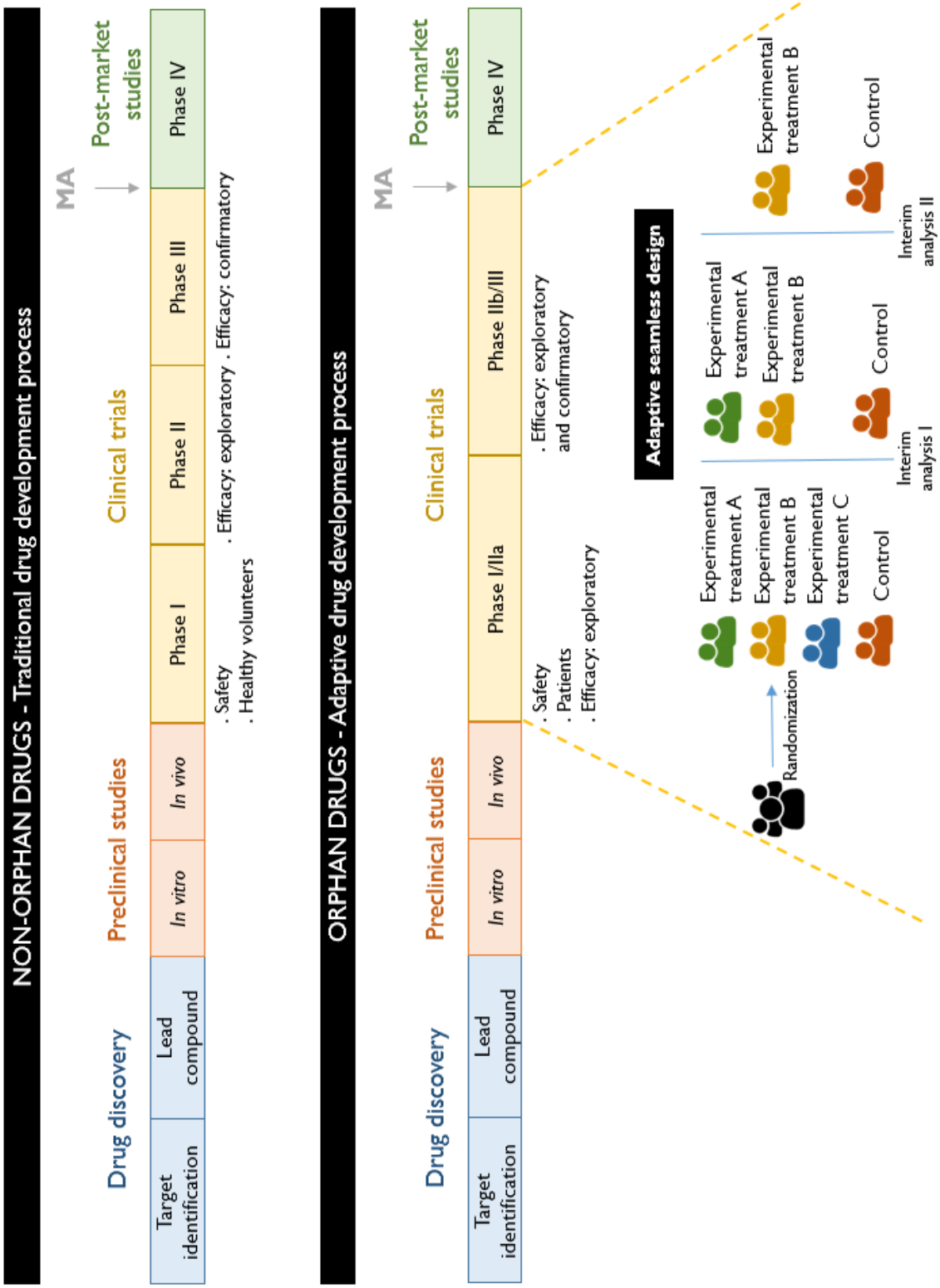

Figure 4: Drug development process for orphan drugs and non-orphan drugs. Focus on clinical phase. Adapted from source [39].

3.4. BIOMARKERS

Biomarkers can be defined as any characteristic that can be measured in the body to evaluate biologic and pathogenic processes, as well as predict the incidence of an endpoint in a therapeutic intervention and quantify treatment responses [44].

With appropriate biomarkers that measure accurately and reproducibly the disease, the ambiguity of clinical studies in rare diseases field could be overcome. Particularly important to conduct trial progression, biomarkers could be the best way for obtaining answers about the evolution of these diseases. Biomarkers can be found in locations fairly distant from their production site and they can be detected in body fluids, hence their importance in giving us useful information for diagnostic, progression or amelioration of a disease stage. This information could be obtained even for inaccessible organs, such as the brain, without using an invasive strategy. Biomarkers are particularly important in cases where data about disease progression and therapeutic effectiveness are limited and hard to obtain, which is the case for brain diseases [45].

Depending on their category, biomarkers can be used with multiple applications as an emerging tool for clinical drug development. Biomarkers can be classified as: surrogate, pharmacodynamic, predictive or prognostic. Biomarkers classification depends on the clinical context in which they are used and the same biomarker can fall under different categories at the same time [8].

Specifically, surrogate biomarkers can evaluate drug efficacy as they can be used as clinical trial endpoints and they can be useful to predict a clinical benefit. For example, in amyotrophic lateral sclerosis (ALS), the creatinine/cystatin C ratio is a surrogate biomarker that helps to evaluate if an experimental intervention is working or not. The decreased levels of creatinine/cystatin C ratio indicates an increase on the severity of ALS patients [8] [46] [47] [48].

The interaction between the experimental therapeutic intervention and the target pathway can be verified by pharmacodynamic biomarkers, supporting validation of clinical data. These biomarkers demonstrate the targeted therapeutic activity and help to make decisions about dose selection. For instance, the urinary level of glycosaminoglycans is a pharmacodynamic biomarker that measures the effect of enzyme replacement therapy for patients with mucopolysaccharidosis type I [8] [49].

For some clinical trials with a heterogeneous population, predictive biomarkers can reduce the intra and inter-individuals variances that hinder result extrapolation [32]. For this reason, they help to identify patient populations for a certain therapeutic intervention. As clinical researchers have usually a poor knowledge of most rare diseases pathophysiology, these biomarkers facilitate rare diseases diagnosis and allow potential subject identification to clinical trials. For instance, in clinical studies of Duchenne Muscular Dystrophy (DMD), an elevation in serum miRNA biomarkers (miR-1, miR-133 and mirR-20) can be observed for patients with this condition, which can make these molecules possible predictive biomarkers [50].

In certain clinical trials for rare conditions, it is relevant to evaluate the disease stage. The progression or alleviation of disease may reflect divergences in the targeted pathway. Using this rationale, prognostic biomarkers are used to provide this clinical information, evaluating the rate of disease evolution. For example, high serum levels of transforming growth factor-β (TGF-β) in Marfan syndrome predict cardiovascular events that suggests disease progression [51].

The quantitative information provided by biomarkers leads to health outcomes prediction and evaluation, allowing reduction of time in clinical development phase [48] [52]. Biomarkers could be an interesting option to be used for research and development by pharmaceutical companies as they can present a faster and cost-effective alternative to clinical outcomes. Moreover, they could be used to increase the diagnostic tools available to detect a rare disease condition and, by doing so, increasing the number of persons included in the target population. The use of biomarkers increases the patients' endpoints and outcomes report, allowing the capture of more information to incorporate in the clinical study analysis and conclusions [53].

Investing in this area would allow for more biomarkers to be discovered, which can potentially accelerate the clinical development process in rare diseases. New biomarkers would allow some therapeutic candidates to advance to clinical trials phase, something that often is not achieved because there is no way of evaluating the response of these new treatments.

3.5. GENETIC AND BIOTECHNOLOGICAL TOOLS

Most rare diseases have a genetic origin and 50% of rare monogenic disorders have been identified at a rate of 250 new diseases every year [32] [54]. Characterization of these diseases has been facilitated by recent advances in genomics [6]. With this knowledge, it is possible to do gene-based discovery (Table 1) [55].

In clinical phase, genetic tools can provide an excellent starting point to translational medicine by allowing the selection of the patients that are expected to respond to treatment from the very beginning, avoiding failures in clinical trials [56]. Specifically, in oncologic research, initial sequencing of the cancer cell DNA may reveal a mutation for which low or no response to experimental intervention is predicted. Consequently, this patient is considered a screening failure that cannot be included in the clinical trial. For instance, in PROFILE trial for Non-small Cell Lung Cancer (NSCLC) patients, one of the inclusion criteria was to have a rearrangement in the anaplastic lymphoma kinase (ALK) gene [57] [58]. Thus, the clinical trial begins with a selected population, as it happens in some of the previously mentioned study designs like enriched enrolment and randomized withdrawal design. Therefore, genetic tools contribute to the improvement of the clinical research and facilitate the implementation of certain study designs.

The heterogeneity of genetic rare diseases increases the challenges in developing effective treatments. To avoid this, the use of accurate genetic tools enables the development of therapeutics based in the disease's genetic origin [8]. Following the progresses of biotechnological methods, research for new treatments for this kind of diseases seems to be promising, pursuing successful examples like Enzyme Replacement Therapies (ERTs) and antibody therapeutics [8]. A reference should be also made to gene therapy, a therapy that provides targeted delivery of nucleic acid material to treat a specific disease caused by a mutant or missing gene [8]. Thus, gene therapy is expected to bring better news for almost of patients with rare diseases as their diseases have usually a genetic cause [8] [59].

Table 1: Examples of therapeutics developed using gene-based discovery and new therapeutic approaches using genetic and biotechnological tools in rare diseases. Adapted from source [8] [55].

Rare disease	Gene	Treatment developed using gene-based discovery	Therapeutic approach to be developed	Ref.
Fibrodysplasia ossificans progressiva	Activin A receptor type I	Activin A antibody	RNAi approach	[60] [61]
Cystic fibrosis specific genetic mutations as in the CFTR gene	Cystic fibrosis transmembrane conductance regulator	Ivacaftor Lumacaftor	Gene therapy (plasmid DNA encoding the CFTR gene complexed with a cationic liposome)	[62] [63]
Pompe's disease	Glucosidase alfa acid	Alglucosidase alfa	Gene therapy (viral vector with the GAA gene targeted to the liver)	[64] [65]
Brown–Vialetto–Van Laere syndrome	SLC52A3 and SLC52A	Riboflavin	-	[66]
Duchenne muscular dystrophy	DMD gene	Exon skipping using antisense oligonucleotides	Gene therapy (AAV mediated delivery of microdystrophin)	[67] [68]

AAV – Adeno-Associated Virus; CTFR – Cystic Fibrosis Transmembrane Conductance Regulator; DMD – Duchenne Muscular Dystrophy; GAA - Glucosidase Alfa Acid; SCL – Solute Carrier

Table 1 shows some examples of how genetic tools have paved the way for the pharmacogenomics era and, consequently, to personalized medicine. This means that studying the drug response associated with each patient's genome is an important translational step to achieve the idealized adaptation of science to individual needs. Particularly relevant for rare conditions, personalized medicine allows a better understanding of disease by integrating several individual pieces of information, like genetic predisposition, cellular phenotype and personal environment [17]. Genetics and biotechnological tools are crucial for the development of targeted therapies that can benefit rare disease patients, as it will be further discussed in the next chapter.

4. RARE DISEASES: CLINICAL THERAPEUTIC APPROACHES

4.1. RARE DISEASES BY THERAPEUTIC AREA

Since the enactment of legislation to support rare diseases research, the development of orphan drugs has increased in the most varied areas (Fig. 5).

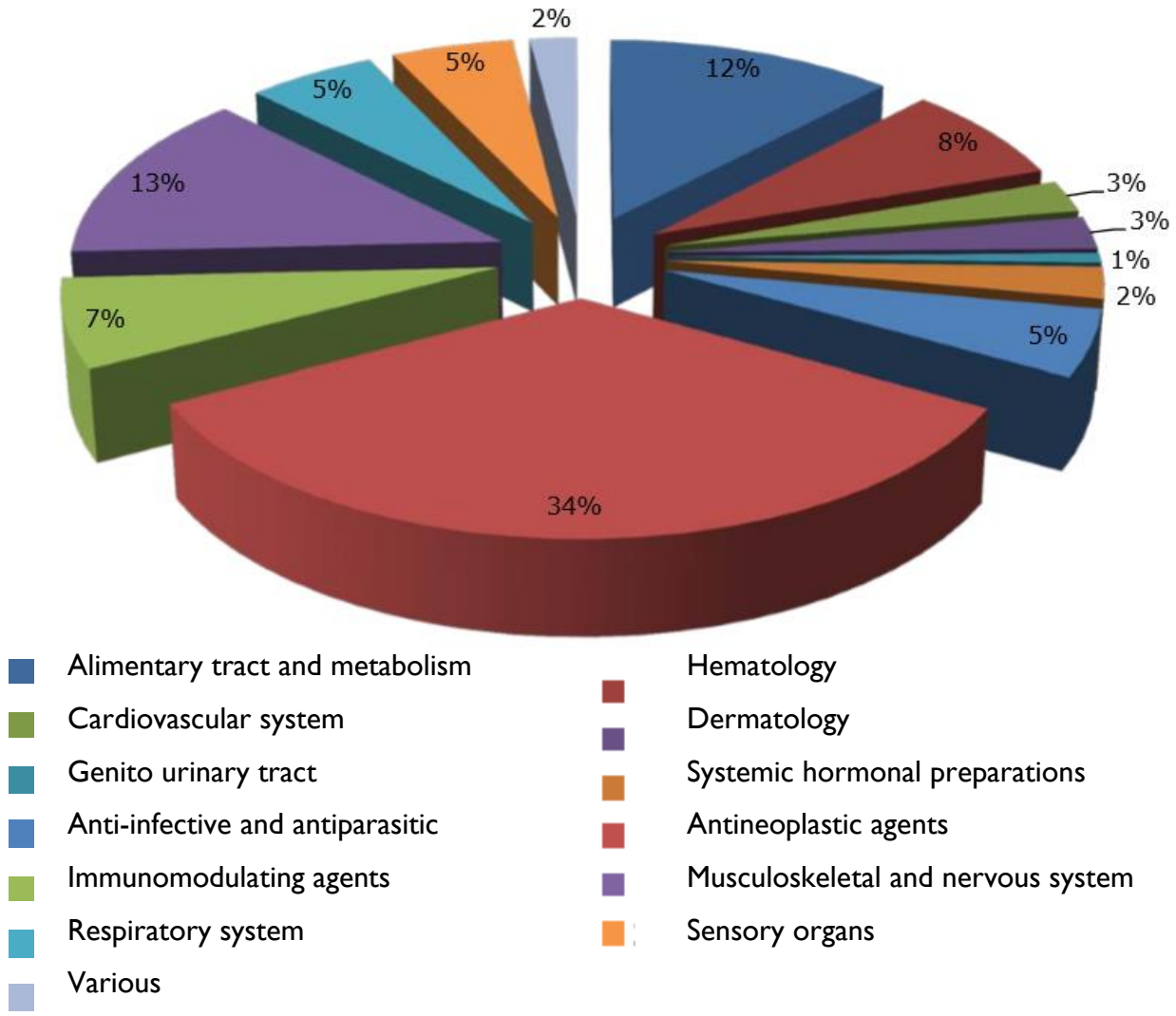

Figure 5: Distribution of positive opinions given for orphan designation requests by EMA according to therapeutic area, from 2000 to 2017. Reproduced from source [127].

According to the analysis made to the positive opinions given by European Medicines Agency (EMA) to orphan designation requests from 2000 to 2017, antineoplastic agents come out as the most appealing area for investment, comprising 34% out of the total number of requests (Fig. 5). The second most promising therapies are those for musculoskeletal and nervous system diseases. Other relevant areas that seem to be of interest for applicants are the alimentary tract and metabolism diseases and hematology. This

analysis shows the evolving trends in the development of innovative therapeutic approaches for rare diseases research, that is known for its lack of therapeutic alternatives.

4.2. RARE CANCERS

A specificity of rare cancers is that they are defined by their incidence rather than their prevalence. While prevalence takes into account the number of patients living with a disease at a certain moment in time, incidence looks at the number of new diagnosis for a specific disease in a determined year. As prevalence is influenced by survival, it is considerate a limited definition to use in rare cancers where the patient's lifespan is decreased. For instance, the European Society for Medical Oncology (ESMO) defines rare cancers as conditions with an incidence of fewer than 6 per 100 000 individuals per year. In the USA, a cancer is considered rare if it has an incidence of fewer than 15 per 100 000 persons per year [69] [70].

Rare cancers are one of the most studied rare diseases, making it easier for clinical researchers to collect quality data on the disease's history. Therefore, drugs for these diseases have a higher chance of success in obtaining orphan drug designation in comparison to drugs for other rare diseases [7]. However, these cancers are still challenging from a clinical standpoint. Apart from the already mentioned difficulties of rare diseases research, researchers also have to take into account all the increasingly smaller molecular genetic subsets expressed frequently in oncologic pathologies [69]. Each one of these molecular disease subsets within the same overarching cancer classification can be considered distinct diseases. This creates the opportunity to develop new targeted treatments that match the molecular subtypes classification with a personalized treatment approach. Thus, the outcomes obtained by applying a targeted therapy would be better than using the same therapeutic approach to treat an unselected oncologic patient. For instance, patients with a rare NSCLC called ALK- rearranged NSCLC, showed a significative regression with crizotinib, an ALK inhibiting treatment [69] [71].

Orphan tumors demonstrate some of the highest therapeutic response rates because, usually, the identification of the main driver of the tumour transformation is possible. Therefore, some of the most successful therapeutic interventions in the oncologic field occurred in rare cancers (Table 2) [69].

One of these successful cases is the treatment for chronic myeloid leukemia, a hematological cancer, that increased the expected survival of patients from 6 to 22 years.

The main driver of this therapeutic revolution was the identification of breakpoint cluster region-abelson (BCR-ABL) kinase as the molecular target for this disease and, consequently, the use of imatinib (BRC-ABL kinase inhibitor). Survival of patients increased, even more, when imatinib was used in an early disease stage and when second generation BRC-ABL kinase inhibitors as nilotinib and dasatinib were identified, allowing to overcome possible resistance to imatinib. Treatment of chronic myeloid leukemia may be an example of the application of personalized therapy for cancer, used to prevent disease progression [69].

Table 2: Examples of therapeutic approaches used for rare cancers according to the identified targets. Adapted from source [69].

Rare cancer	Target gene mutation	Therapeutic approach
Acute promyelocytic leukemia	PML-RAR rearrangement	All-trans retinoic acid
Chronic myeloid leukemia	BRC-ABL	Imatinib, nilotinib, dasatinib
Hairy-cell leukaemia	BRAF	BRAF inhibitors p.e. 2-chorodeoxyadenosine
Inflammatory breast cancer	ALK amplification	Crizotinib

ALK: Anaplastic Lymphoma Kinase; BRAF: B-raf; BRC-ABL: Breakpoint Cluster Region-Abelson; KIT: transmembrane Tyrosine Kinase; PML-RAR: Promyelocytic Leukemia-Retinoic Acid Receptor

Similar results can be achieved for solid tumors, using targeted therapy according to the molecular profile of the tumour. For example, inflammatory breast cancer is a rare condition that usually does not have the desired therapeutic response to standard treatment. This divergence can be related to genetic features such as a high level of TP53 mutations, or the major prevalence of triple-negative form for this rare condition. Furthermore, these differences in treatment responses may be due to the specific molecular characteristics of this cancer, such as the ALK gene amplification found in most of these rare condition cases. One possible therapeutic solution are ALK inhibitors, already being used for the rare form of NSCLC mentioned above, with the added benefit that active mutations in ALK gene are not as common as they are for the NSCLC case referred (Fig. 6) [72].

Targeted therapies tailored to molecular sub-classifications can be even more effective for solid tumors due to their intratumor heterogeneity, where different molecular profiles for the same tumor are observed in the same individual. Once again, in the case of breast cancer, it may be necessary to repeat a biopsy to identify new possible molecular tumor changes according to disease progression. This can be overcame using effective

therapeutic combinations in accordance with the identified molecular disease subset [73] [74].

The shift from the current scientific approach to one that targets specific carcinogenesis pathways, at a smaller and more detailed scale, avoids tumor proliferation without damaging the normal cells around it. This allowed the development of breakthrough therapeutics in the oncologic field, such as the blockade of programmed death 1 (PD-1) immunotherapy and antibody-linker-chemotherapy conjugates [69].

Therefore, because of their specific characteristics, rare cancers are important drivers in the development of tailored therapeutic approaches and innovative therapies. Furthermore, the development of new diagnostic tools at a molecular level increased pathophysiology knowledge, which is especially relevant in the rare diseases field, as it offers a more precise diagnosis and facilitates medical decision making.

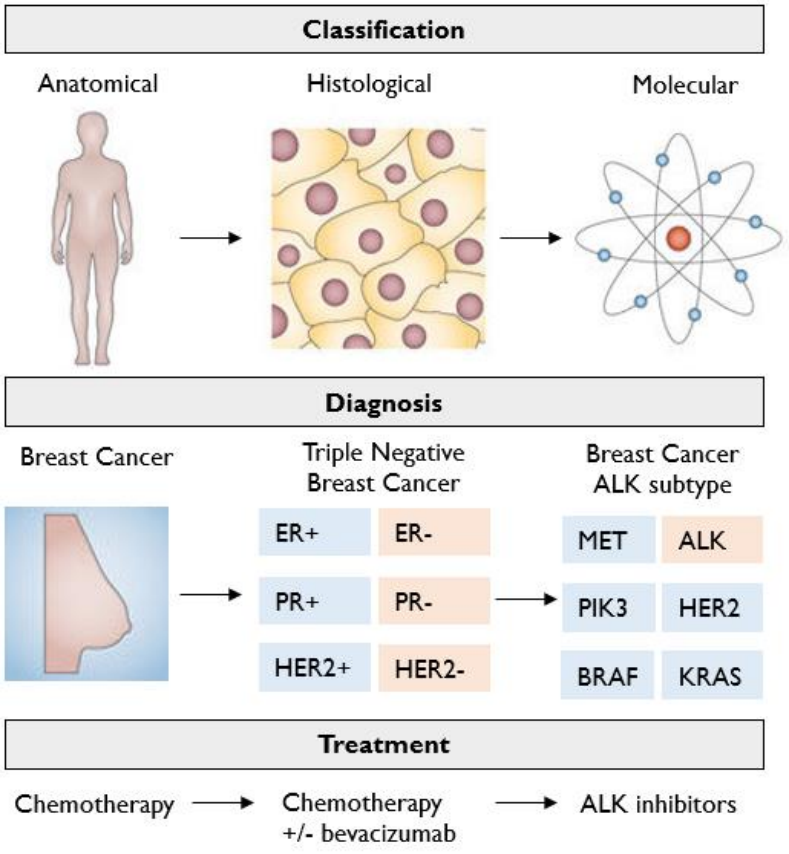

Figure 6: Evolution of cancer classification, diagnosis and treatment according to the molecular profile subsets. Adapted from source [69].

4.3. RARE DISEASES IN MUSCULOSKELETAL AND NERVOUS SYSTEM

There are more than 600 diseases that affect the nervous system and, when the brain is affected, a wide range of body functions can be compromised. Particularly, there is a wide range of diseases affecting the nervous system in a neurodegenerative way. This kind of diseases usually have an hereditary feature and they are characterized by a late diagnosis, chronic clinical progression and progressive neuronal loss in specific brain regions [75].

An example of a known rare neurodegenerative disease is amyotrophic lateral sclerosis (ALS) which is characterised by progressive degeneration of motor neurons that are present in the central nervous system. This disorder causes degeneration of spinal motor neurons which causes secondary denervation and muscle loss (thus called amyotrophic), and the degeneration of corticospinal motor neurons and descending axons packed in the lateral spinal cord (thus called lateral). Therefore, its clinical manifestations are the result of an ineffective communication between spinal motor neurons and corticospinal neurons, which leads to loss of control of skeletal muscles. As a typical neurodegenerative disease, it first appears at one limited location and then spreads slowly to other regions. Initial symptoms include cramping or muscle weakness with progressive worsening until skeletal muscle paralysis. This disorder usually appears in adulthood and it is difficult to achieve a final diagnosis [76].

Several mechanisms are involved in the pathophysiology of ALS: mutations in the genes responsible for quality control of cellular proteins, such as the superoxide dismutase (SOD) 1 gene; hyperactivation of microglia, that produces oxidative stress and leads to neuroinflammation; decreased levels of energy carried by oligodendrocytes to motor neurons; excitotoxicity caused by decreased glutamate uptake; defects in cytoskeletal and modified axonal transport and impairment in RNA metabolism [76]. Due to the multiple therapeutic targets available and an incomplete comprehension of this disease's pathological mechanisms, there is currently no cure for ALS. The available therapeutics can only slow disease progression. For example, approved in 1995 by FDA, riluzole (Rilutek®) reduces extracellular glutamate levels by blocking its release and protecting motor neurons from excitotoxicity [77] [78]. In 2015 in Japan and in 2017 in the USA, edavarone (Radicut®/Radicava®) was approved as an ALS therapeutic agent that decreases oxidative stress and consequently neuroinflammation [79]. This molecule, which used before for stroke treatment, went through several clinical trials that were efficiently adapted in order to demonstrate the efficacy and security of edavarone in ALS, especially in the early stages of

the disease (Table 3) [80] [81]. Other treatments are still in clinical trial stage, like masitinib, that has proven to be able to decrease the microglia hyperactivation through inhibition of tyrosine kinase, an enzyme involved in the neuroinflammatory process [82]. New approaches to ALS are aiming at combining therapies, taking into account the heterogeneous nature of this disease or targeting genes that are known to be involved in pathophysiologic mechanisms. One of the most promising approaches to treat ALS is antisense therapy. This approach uses small portions of nucleic acid materials that binds to specific portions of messenger RNA and bocks the translation of proteins involved in ALS. In this case, the objective is to modify the activity of SOD1 mutated genes and stop the production of non-functional proteins involved in ALS [82]. Also, the C9ORF72 gene was identified as a possible target using this therapeutic approach [76] [82]. Apart from that, the idea of using gene therapy to replace the affected genes in cells that support motor neurons activity, like glia cells, is getting more and more traction in ALS research. [82].

Table 3: Examples of current therapeutic developments for ALS treatment with evidence based in clinical trials.

Therapeutic agent	Clinical trial objective	Primary endpoint	Action/ Target	Stage	Ref.
Riluzole (Rilutek®)	Efficacy and safety of riluzole in patients with ALS	Survival and rates of change in functional status	↓glutamate levels	Approved	[83]
Edaravone (Radicut®/ Radicava®)	Efficacy and safety of edaravone in patients in early stage of ALS	Revised ALS Functional Rating Scale (ALSFRS-R) at 24 weeks	↓oxidative stress	Approved	[81]
Masitinib	Efficacy and safety of masitinib in combination with riluzole in ALS patients	Changes in ALSFRS-R	↓glia hyper-activation	Phase II/III	[84]
Antisense oligo-nucleotide (ISIS 333611)	Safety, tolerability and pharmacokinetic of ISIS333611 after intrathecal administration in patients with SOD1-related familial ALS	Dose-escalation to test safety, tolerability, and pharmacokinetics of experimental drug	↓non-functional SOD proteins	Phase I	[85]

ALS: Amyotrophic Lateral Sclerosis; ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale; SOD: Superoxide Dismutase

There are other nervous system diseases that cause a fast loss of neuronal function. For instance, spinal muscular atrophy (SMA) is a rare genetic condition that also affects motor neurons and it is one of the major genetic causes of death during childhood. This disorder is characterized by a defective or non-existent survival motor neuron (SMN) gene that is responsible for the production of SMN protein. This protein is essential for the survival of cells that are responsible for muscle control. Thus, clinical manifestations for this disease include loss of muscle strength and control, muscle atrophy and difficulties in basic motor functions like breathing. The severity of this neuromuscular dystrophy depends on the type of SMN gene that is damaged: it is more severe if it affects the SMN1 gene and intermediate or mild if the gene affected is the one for SMN2. As the SMN1 gene produces a greater amount of SMN protein than the SMN2 gene, patients with SMA usually have more than one copy of the SMN2 gene to compensate for low production of SMN protein [86]. To restore the levels of SMN protein, a novel therapeutic was developed targeting the SMN2 gene: nusinersen (Spiranza®). It is an antisense oligonucleotide that increases the amount of SMN functional protein in SMA patients. To reach motor neurons, nusinersen is administered directly into the cerebrospinal fluid, through intrathecal injection. Despite the invasive administration route, this therapeutic approach improved the average life expectancy of SMA patients. Future trends point towards the development of therapeutics that allow production of SMN protein from a functional SMN1 gene, as it is presented in Table 4 [86] [87].

Table 4: Examples of current therapeutic developments for SMA treatment with evidence based in clinical trials.

Therapeutic agent	Clinical trial objective	Primary endpoint	Action/ Target	Stage	Ref.
Nusinersen (Spiranza®)	Efficacy and safety trial of nusinersen in infants with SMA	Motor-milestone response and event-free survival	SMN2 splicing	Approved	[88]
AVXS-101	Safety and efficacy of gene therapy in SMA patients	Time from birth to requirement of ≥16hour respiratory assistance; Dose-escalation	SMN1 replacement	Phase I	[89]

SMA: spinal muscular atrophy; SMN: survival motor neuron

4.4. RARE METABOLIC DISEASES

Metabolic diseases can be divided into three main groups. The first group includes diseases caused by the accumulation of a toxic substances due to a defective function of an enzyme or transport protein. The second group comprises disorders characterized by a cellular dysfunction cause by a defect in energy production mechanisms, that eventually causes metabolic crises induced by stress. The third group includes all conditions in which a progressive dysfunction caused by the storage of large molecules in the cellular organelles is observed [75].

Lysosomal storage diseases are included in the third group. These rare metabolic diseases are related to inborn errors in genes that are responsible for inadequate lysosomal function. They represent more than 70 rare inherited diseases that affect both children and adults. Depending on the defected gene, there will be an accumulation of different macromolecules for each disorder with different causes (Fig. 7). This genetic error can be expressed on the membrane and transport lysosomal proteins or on the lysosome enzymes that usually will result in chronic diseases. Furthermore, as lysosomes are the organelles responsible for catabolic cell activity and they are present in all nucleated cells, an impairment in their function leads to the coexistence of pathological conditions which narrow down therapeutic options for these conditions [90].

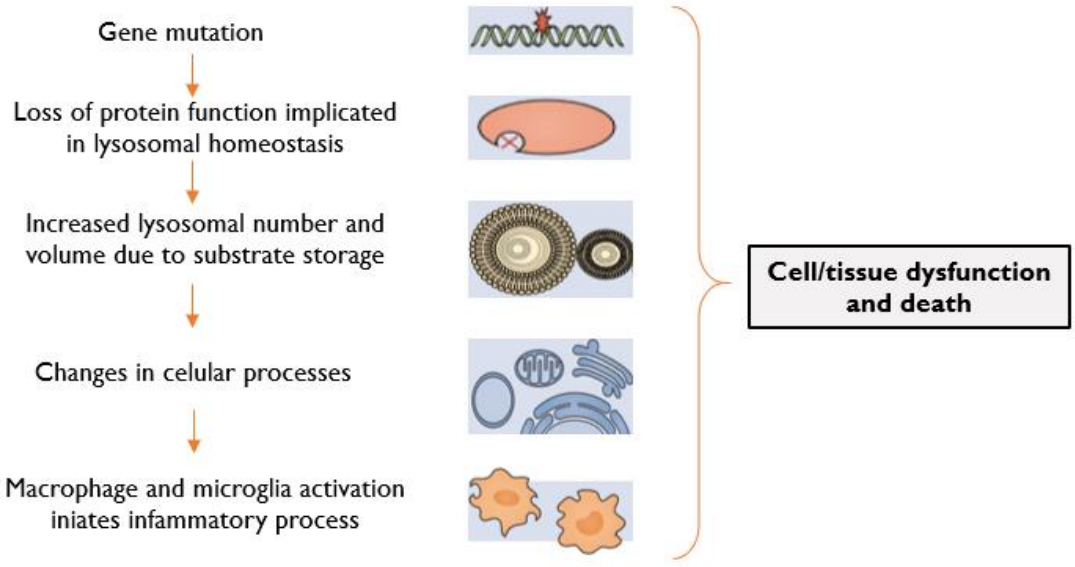

Figure 7: Common pathological pathway in lysosomal storage diseases. Adapted from source [90].

An example of a lysosomal storage disease is Gaucher disease. It is characterized by an error in the gene that encodes for the β-glucocerebrosidase (GBA), a lysosomal hydrolase. This enzyme is responsible for the cleavage of the glycolipid membrane molecule, glucocerebroside, into glucose and ceramide [91]. The ineffective activity of this hydrolase causes the accumulation of glucocerebroside in the organelles of the cell, thereafter designated Gaucher cells. The most common clinical manifestations of this disease are hepatosplenomegaly, hematological deregulations and bone lesions [91].

One of the possible therapeutic approaches for this disease is enzyme replacement therapy (ERT). As the name suggests, this therapy replaces the missing/defective enzyme that causes the disease, such as GBA in Gaucher disease case. The first ERT to be marketed was alglucerase (Ceredase®), a synthetic and modified form of GBA. This discovery increased the investment in ERT due to its success [90]. Since then, other analogous enzymes were developed for Gaucher disease like imiglucerase (Cerezyme®), taliglucerase (Elelyso®) and velaglucerase (VPRIV®) [91]. However, despite the positive results of these biologics, this therapeutic approach has its own limitations, such as: difficulty to reach tissues like bone and brain; adverse reactions due to patient's sensitivity and use of invasive administration routes such as intravenous. [90].

Another less invasive therapeutic strategy for Gaucher disease consists in decreasing the excessive amount of accumulated glucocerebroside, by reducing the amount of substrate of catabolism, the so-called Substrate Reduction Therapy (SRT). For example, miglustat (Zavesca®) and eliglustat (Cerdelga®) are inhibitors of the glucosylceramide synthase, an enzyme involved in the biosynthesis of glycolipids. The inhibition of this enzyme, leads to a decrease in the biosynthesis of glucocerebroside, improving treatment outcomes [90]. Current therapies on Gaucher disease are summarized on Table 5.

Attempts to apply gene therapy in Gaucher disease have also been made. A phase I study tried to transfer the GBA gene into peripheral blood stem cells, using a retroviral vector. Then, the genetically modified cells were reintroduced in patients through an autologous transplant and the clinical effects were measured. However this study didn't follow through the following phases due to lack of evidence of clinical benefit and low-level gene marking observed *in vivo* [92] [93].

Table 5: Examples of current therapeutic developments for Gaucher disease. Adapted from source [90] [91].

Therapeutic agent	Action/ Target	Stage
Alglucerase (Ceredase®)	ERT for GBA	Approved on 1991
Imiglucerase (Cerezyme®)		Approved on 1994
Velaglucerase (VPRIV®)		Approved on 2010
Taliglucerase (Elelyso®)		Approved on 2012
Miglustat (Zavesca®)	SRT for glucosylceramide synthase	Approved on 2002
Eliglustat (Cerdelga®)		Approved on 2015

ERT: Enzyme Replacement Therapy: GBA: β-Glucocerebrosidase; SRT: Substrate Reduction Therapy

Within the lysosomal storage diseases group, there are also the mucopolysaccharidoses disorders. These rare diseases are caused by the absence or deficiency in enzymes that break down the glycosaminoglycans, mainly present in cells that constitute the skin, bones, cartilage and cornea [94]. Without the ability to cleave the complex polysaccharides, they will accumulate in the cell environment. Clinical manifestations and disease severity varies according to the type of mucopolysaccharidosis. There are 7 different forms of mucopolysaccharidoses, that are presented in Table 6, classified according to the enzyme that is lacking or not working properly.

Mucopolysaccharidosis VII, also known as Sly syndrome, is characterized by a faulty activity of the β-glucuronidase enzyme due to mutations in the GUSB gene. Clinical manifestations for this disease are noticed during early childhood and include cognitive impairment, growth retardation and skeletal disabilities [95].

This is an extremely rare condition and, until recently, no treatment was available. In 2017 the FDA approved an ERT for this disease, vestronidase alfa (Mepsevii®) [96] [97]. This approval was supported by the results of a clinical trial with expanded protocols that

included 23 patients aged from 5 months to 25 years old. This clinical trial's objective was to demonstrate the safety and efficacy of vestronidase alfa for Sly syndrome patients. Patients were given vestronidase alfa at doses of up to 4 mg/kg once every 2 weeks, by intravenous administration, for up to 164 weeks. The primary efficacy endpoint assessed was the 6-minute walk test at week 24, which revealed a significant improvement in 10 out of the 23 patients. After this, the follow-up period demonstrated an improvement in 3 patients and stabilization in the remaining patients [98].

This approval represents a big achievement for these patients as they now have therapeutic options that go beyond symptomatic and supportive therapy.

Table 6: Mucopolysaccharidosis classification according to the missing/defective enzyme. Adapted from source [95].

MPS	Syndrome	Missing/ defective enzyme	Therapeutic agent	Stage
I	Hurler /Scheie	α-L- iduronidase	Larodinase (Aldurazyme®)	Approved on 2003
II	Hunter	Iduronidate-2-sulfatase	Idursulfase (Elaprase®)	Approved on 2006
III (type a, b, c or d)	Sanfilippo	Heparan-N-sulfatase α-N-acetylglucosaminidase α-glucosaminidase acetyltransferase N-acetyl-glucosamine-6-sulfatase	Gene therapy (SGSH gene in AAV) - MPS III type a and b	Phase I/II
IV (type a or b)	Morquio	N-acetyl-galactosamine-6-sulfate sulfatase β -galactosidase	Elosulfase alfa (Vimizin®) - MPS IV type a	Approved on 2014
VI	Maroteaux-Lamy	N-acetylgalactosamine-4-sulfatase	Galsulfase (Naglazyme®)	Approved on 2005
VII	Sly	β-glucuronidase enzyme	Vestronidase alfa (Mepsevii®)	Approved on 2017
IX	Natowicz	hyaluronidase	-	-

AAV: Adeno-Associated Virus; MPS: Mucopolysaccharidosis; SGSH: N-sulfoglucosamine sulfohydrolase

4.5. NEW THERAPEUTIC APPROACHES FOR RARE DISEASES

Despite the limited range of therapeutic options available for rare diseases, new therapeutic approaches developed tend to be more effective as they are designed to achieve molecular targets of these diseases [17]. As it was previously mentioned, research for new therapies has advanced significantly due to advances in scientific tools. Therefore, as it was previously covered, biologics and gene therapy are a reality in relevant therapeutic areas.

Rare diseases are a booster for the development of new therapeutic approaches and innovation strategy. For instance, biologics represent a highly selective therapeutic option for which there is a high volume of investments being made. One of the biologic products approved in 2017 by the FDA was emicizumab (Hemlibra®), a monoclonal antibody developed to treat hemophilia A. This antibody mimics the function of a protein essential in blood coagulation, factor VIII. This is the missing/defective blood coagulation cascade factor in patients with this rare condition [96] [99]. Another biologic product approved last year was nonacog beta pegol (Rebinyn®), a recombinant coagulation factor for the treatment of hemophilia B. Rarer than hemophilia A, this type of hemophilia is characterized by the absence of coagulation factor IX [99].

Also, gene therapy showed its own progress in the last few years. Many of the conditions that benefit from gene therapy are rare disorders. If we look back to the approved gene therapies, one thing we notice is that they were approved for use in rare diseases. For instance, in 2012, the EMA approved alipogene tiparvovec (Glybera®) to treat a subset of patients with lipoprotein lipase (LPL) deficiency. As this enzyme is ineffective in patients with this rare condition, catabolism of triglycerides rich lipoproteins is impaired, leading to, for example, severe hypertriglyceridemia that may induce pancreatitis. For these patients, Glybera® can be used. This is an adeno-associated virus (AAV) vector local *in vivo* therapy, whose genetic material encodes a functional LPL and it is applied in several sites of the lower limbs' muscles [100]. Clinical trial data used to support its approval show a decrease in the number of pancreatitis episodes in the long term for patients with this disease [101]. However, in 2017, Glybera® marketing authorisation was not renewed in the European market, the reason being that it failed to decrease triglycerides levels, which had been defined as the primary endpoint to reach [102].

A more positive example came in 2016, when the EMA approved an *ex vivo* gene therapy for the treatment of the severe combined immunodeficiency due to adenosine

deaminase deficiency (ADA-SCID), also known as the bubble boy disease, which is characterised by a failure in the production of functional lymphocytes. Strimvelis® is a therapy that comprises of hematopoietic stem cells from the patient, which after being collected are selected and transduced with a retroviral vector containing the ADA gene and, after selection, the corrected cells are reintroduced into the patient [103] [104]. The clinical trial conducted was aimed at demonstrating the safety and efficacy of Strimvelis®, and it included 12 patients aged between 6 months and 6 years old, for whom there was no bone marrow donor available. After the treatment, the incidence of serious infections decreased in the short and long term. All patients were alive at least 3 years after the treatment [105].

In the USA, the first gene therapy was approved in 2017. This *in vivo* gene therapy is called voretigene neparvovec (Luxturna®) and it represents a treatment for a rare inherited genetic disease that causes loss of eyesight. It was developed using a AAV serotype 2 vector that deliveries a normal RPE65 gene to retinal cells [96]. The clinical trial that demonstrates the efficacy and safety of this gene therapy included 31 patients who were evaluated through a mobility test to assess functional vision. After treatment, all patients had improved their performance in this test, in comparison to the control group. There were no serious adverse events related with the product nor any severe immune response [106]. As this gene therapy is administrated by subretinal injection, it has some advantages: good accessibility to the organ, reduced immune response reactions and low risk of systemic side effects due to compartmentalization. Besides that, the other eye can be used as an internal control for the treatment [107]. Single administration of Luxturna® serves as an example of successful gene therapy, as it fulfils its final objective, which is to treat a disease at its genetic origin [108].

Thus, rare diseases could benefit greatly from gene therapy, as most of them have a genetic cause. This therapeutic approach gives hope to rare disease patients, who can believe in a future where therapeutic options for their conditions go beyond symptomatic treatments to a definitive cure.

5. REGULATORY FRAMEWORK

5.1. ORPHAN DRUG DESIGNATION

Orphan drug designation is given to medicinal products that are used to treat rare diseases, which are defined not only by their prevalence, but also by other criteria like severity of disease, existence of alternative therapeutic solutions and return on investment from drug development and production, according to specific regulatory policies adopted regionally.

In the USA, a drug is considered to be an orphan drug if it was developed to treat a disease that affects less than 200 000 people. However, should this drug be used to treat a disease that affects more than 200 000 people, but its costs of development and production are not expected to be recovered, orphan designation can also be granted [7] [109].

The orphan drug designation was formalized in the USA, in 1983, by the enactment of the Orphan Drug Act (ODA), which is administered by the Office of Orphan Products Development (OOPD) at the Food and Drug Administration (FDA) [110].

Once requested, an assessment for granting orphan drug designation will be performed and, during this evaluation period, the OOPD can raise questions to the applicant. Once a positive decision is issued, the applicant must submit a report on the present and future plans for development of the orphan drug within 14 months and annually thereafter. Depending on this report, the OOPD can decide to maintain or withdraw the orphan drug designation [7].

In Japan, the criteria needed for a drug to obtain orphan designation are: to affect less than 50 000 persons; to treat serious diseases for which no other treatment is available or to have a significant effectiveness or safety when compared to the other treatment options; and evidence for the therapeutic indication with a plausible plan for product development [2].

To encourage the development of medicines for rare diseases, the orphan drug regulation was established in Japan, in 1993, with the revision of the Pharmaceutical Affairs Law (Law145 -10 August 1960) [111].

Orphan drug designation request is evaluated by an independent regulatory agency, the Pharmaceutical Medical Devices Agency (PMDA), which prepares a scientific report. Based on this report, the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) issues

an opinion that is forwarded to the Minister of Health, Labour, and Welfare (MHLW). The MHLW grants orphan drug designation considering PAFSC's opinion. This process can take between 3 and 6 months and the final decision regarding orphan drug designation is published in the Government Gazette [7].

In the EU, orphan designation is given to a medicinal product that diagnoses, prevents or treats a life-threatening or chronic debilitating disease that affects no more than 5 in 10 000 persons; or a medicine for a life-threatening or chronic debilitating condition that, without incentives, would not generate enough return to justify the investment. Apart from this, orphan designation is given to a medicinal product that demonstrates a significant benefit for those affected, in other words, medicinal products with advantages for patients with conditions for which no other suitable methods of diagnostic, prevention or treatment are available [109] [112].

It was only in 1999 that the EU gave the first steps towards adopting legislation for orphan drugs, with Regulation EC N° 141/2000, encouraging pharmaceutical companies to invest in orphan drug development and production. It defined the orphan drug designation procedure and created the Committee for Orphan Medicinal Products (COMP), a committee of the European Medicine Agency (EMA). Other legislation updates were made throughout the years to set up the implementation of the criteria for orphan drug designation. One specificity of the EU legislation is that a centralised procedure is mandatory. Centralised procedure means that only one marketing authorisation is issued for an orphan drug to be commercialized in all countries of the EU [113] [114].

To request orphan drug designation in the EU, the applicant may use the common EMA/FDA application form which facilitates communication between both regulatory authorities. There is also the opportunity to do a parallel submission with the Japanese regulatory authorities. In the pre-submission phase, that is highly recommend but not mandatory, there are two coordinators: a member of COMP and a scientific administrator of EMA. This stage allows dialog and discussion regarding information to be included in the application, to ensure a successful submission. Once submitted, the application goes through a validation process. Once this is done, orphan drug designation is evaluated by COMP in a period of no longer than 90 days. During this period questions can be raised, or further data can be requested. The final opinion of COMP is communicated to the European Commission (EC), which has 30 days to make a decision. If a positive opinion by COMP is followed by orphan drug designation granting by the EC, this must be documented in the Community

Register of Orphan Drugs. Annually, a report about the orphan drug development progress must be presented to the EMA [7] [115].

Despite all the differences in definition and regulatory policies across the world (Fig. 8), especially in the number of patients needed for a disease to be considered "rare", and in the orphan designation request process, there is a consensus to find a global definition for orphan drug. This would be the first step to the standardization of orphan drug development processes [7].

It is relevant to highlight that after obtaining orphan drug designation, a medicinal product doesn't have an authorisation to be commercialized. Instead, it gives certain regulatory supports and benefits to the pursuant of the marketing authorisation that will be further discussed in the next topic.

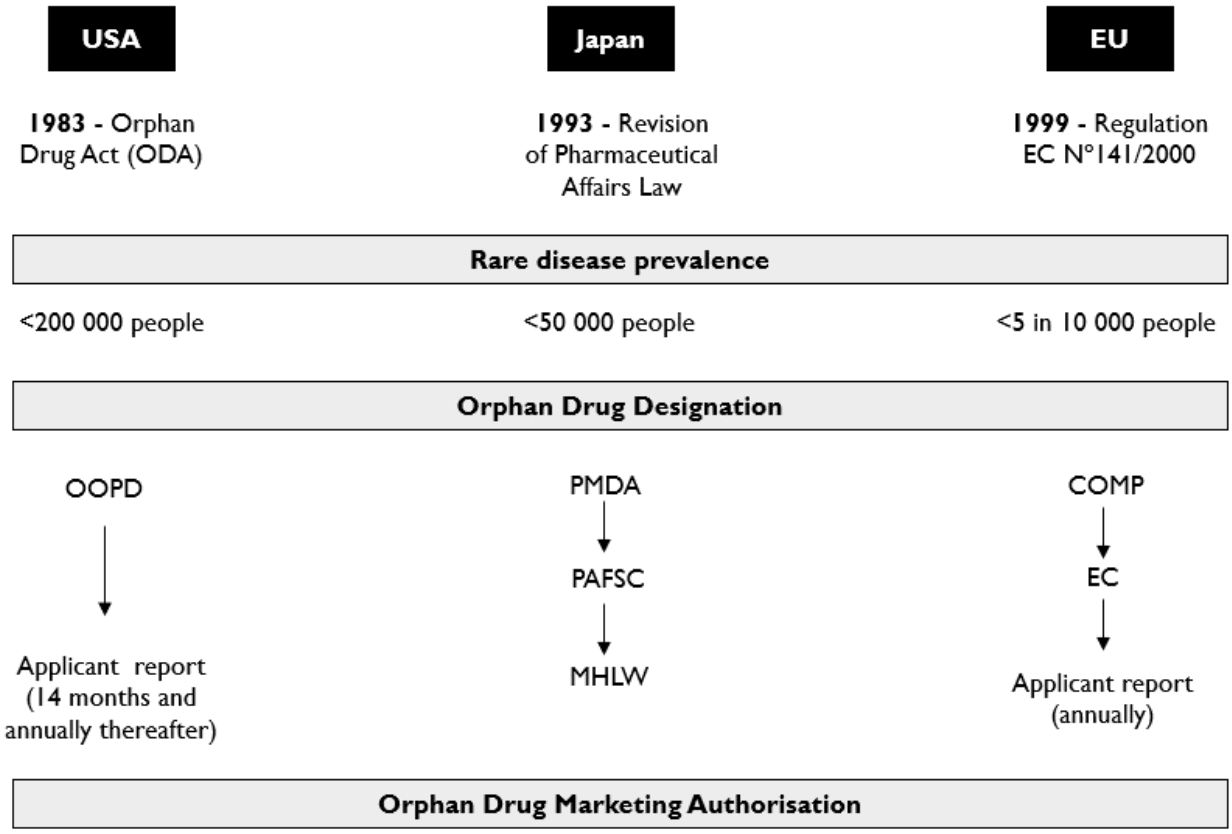

Figure 8: Main differences in the regulatory policies to request an orphan drug designation between USA, Japan and EU. Adapted from source [7].

COMP: Committe for Orphan Medicinal Products; EC: European Commission; EU: European Union; MHLW: Ministry of Health, Labour and Welfare; ODA: Orphan Drug Act; OOPD: Office of Orphan Products Development; PMDA: Pharmaceuticals and Medical Devices Agency; PAFSC: Pharmaceutical Affairs and Food Sanitation Council; USA: United States of America

5.2. INCENTIVES FOR ORPHAN DRUGS RESEARCH AND DEVELOPMENT

The investment for the development of medicinal products for rare diseases might not be recovered by the expected sales revenue without government incentives, as the size of the potential market is small. Thus, it does not seems an attractive area for pharmaceutical companies to invest [7]. However, most treatments available for rare diseases are only symptomatic with no cures yet available. The urgency in finding solutions for these patients has led governments and regulatory authorities worldwide to try to stimulate research and development of orphan drugs, with specific legislation and policies which represent certain incentives (Table 7) [5].

To benefit from the incentives granted by regulatory authorities, orphan drug designation must be obtained. One of the incentives provided by almost all regulatory authorities is scientific advice or/and protocol assistance that grants a specific type of scientific guidance for orphan medicines developers [7]. Scientific advice will help sponsors to get information on the types of studies needed to demonstrate efficacy, safety and quality of their medicinal products. With this support, chances of success are higher because the appropriate tests are done from the beginning, which decreases the probability for questions to be raised in the next phases, which could delay the clinical development process. It is important to note that scientific advice is done prospectively, with the objective of delineating development strategies and not focusing on the evaluation for marketing authorisation [116].

Specifically, protocol assistance will allow sponsors to obtain more information about their medicine, including information about the significant benefits for the designated orphan indication. This information is relevant because it can demonstrate clinical similarity or superiority over other medicines, which can grant market exclusivity [116].

Another regulatory tool to promote orphan drug development is giving extra years of market exclusivity to the marketing authorisation holder. This protects the new orphan drugs from market competition, with small differences between world regions [117].

In the EU and USA, orphan market exclusivity rights relate to the therapeutic indication for which it was granted marketing authorisation. This means that, during this exclusivity period, no other medicinal product with identical characteristics is authorised by regulatory authorities as an orphan drug for the same therapeutic indication. While in the

USA market exclusivity period lasts for 7 years, in the EU this period goes from 10 up to 12 years if the medicine is under a pediatric investigation plan [7] [117].

However, both in the USA and in the EU, it is possible to cease market exclusivity for an orphan drug already authorised if another applicant proves that has a new medicine for the same therapeutic indication that doesn't have the same chemical structure or similar mechanism of action. Furthermore, the new orphan drug must demonstrate clinical superiority, which means that it needs to have better safety or efficacy profiles, providing significative clinical benefits for the patients. Another way to prematurely end the years of market exclusivity of an orphan medicine is to prove that the first applicant has not the capacity for producing enough drug to respond to current market demands. There is also the possibility of reaching an agreement between the first and second applicants, in which the original orphan drug marketing authorisation holder will authorise the second applicant to produce his medicine [7] [118].

In Japan, the concept of market exclusivity is assigned to a re-examination period, which lasts usually 10 years for orphan drugs. During this period, no marketing authorisation application can be filed for the same active substance. This re-examination period works like a constant renewal of a marketing authorisation application and confers protection against market competition [7].

Besides scientific advice and marketing exclusivity, financial incentives are also given to support orphan drugs research and development. These include financial support for clinical and non-clinical research, either by credit and subsidies or by fee reduction or exemption [5].

Another relevant incentive is the creation of funding research programmes. For instance, in the USA there is an Orphan Products Clinical Trials Grants Programme (OPCTGP), promoted by the FDA, that supports clinical development of orphan drugs. This programme encourages mainly small companies and academic groups to move their medicinal products to the clinical phase, with an important scientific and financial support [5] [119]. According to the FDA, this programme has already provided the authorisation of more than 55 orphan drugs in the USA [120]. Other similar programmes are funded by the National Institutes of Health (NIH). In the European case, there are programmes created by the European Commission to support rare disease research that can be found in the EU Framework Programmes (FP) [5]. In Japan, grant programmes for the individual researchers

and small and medium-sized enterprises are provided by the Agency for Medical Research and Development (AMED) and the National Institute of Biomedical Innovation (NIBIO) [5].

With these incentives, orphan drug development becomes an attractive and cost effectiveness business at the same time [5].

Table 7: Comparison of regulatory incentives for orphan drugs development in the USA, EU and Japan. Adapted from sources [5] [111] [7].

Orphan Drugs Incentives	USA	EU	Japan
Scientific advice	. Yes - free	. Yes – until100% fee reduction	. Yes – 30% fee reduction
Marketing exclusivity	. 7 years	. 10 years	. 10 years
Financial incentives	. Tax credit until 50% for clinical trials . Fees reduction for MA submission	. Price and reimbursement incentives for member states . Regulatory fee reduction for small and medium enterprises	. Subsidies up to 50% for clinical and non-clinical research . User fee waivers
Research Programmes	. FDA (OPCTGP) . NIH	. EU FP	. AMED . NIBIO

AMED: Agency for Medical Research and Development; EU: European Union; FP: Framework Programmes; FDA: Food and Drug Administration; MA: Marketing Authorisation; NIBIO: National Institute of Biomedical Innovation; National Institute of Biomedical Innovation; NIH: National Institutes of Health; OPCTGP: Orphan Products Clinical Trials Grants Programme; USA: United States of America

5.3. MARKETING AUTHORISATION STAGE

A marketing authorisation application aims to grant market approval for a medicine based on a full review of quality, safety and efficacy data, including clinical study reports [121]. To submit a market authorisation application for an orphan drug it is necessary to obtain orphan drug designation before [7].

There is no difference between the process for obtaining a market authorisation of an orphan or non-orphan drug in the USA and in Japan, in the sense that it is not necessary

to reassess the orphan drug designation to market a product as an orphan drug, with the exception of the annual reports that are sometimes requested, for instance in the USA [7].

However, in the EU, besides the annual reports, the marketing authorisation applicant must submit a report demonstrating that the orphan drug designation is still valid and should be maintained for that medicinal product. The maintenance of orphan drug designation report must include: the current prevalence of the disease to be prevented, diagnosed or treated or the potential return on investment; the present life-threatening or debilitating nature of the disease; the current existence of other medicinal products available for the condition and, if applicable, a justification of the medicine's significant benefit [118]. The COMP evaluates this request to reconfirm that orphan drug criteria are met and issues an orphan maintenance assessment report in parallel and independently of the evaluation of the marketing authorisation application by the Committee for Medicinal Products for Human Use (CHMP) [7] [118]. The COMP forms an opinion on the orphan drug designation maintenance, following the CHMP positive opinion about the orphan drug marketing authorisation application, that is sent to the EC to confirm and grant marketing authorisation [118]. Even with the reconfirmation or maintenance of orphan drug designation, the orphan drug marketing authorisation may not be granted (Fig. 9).

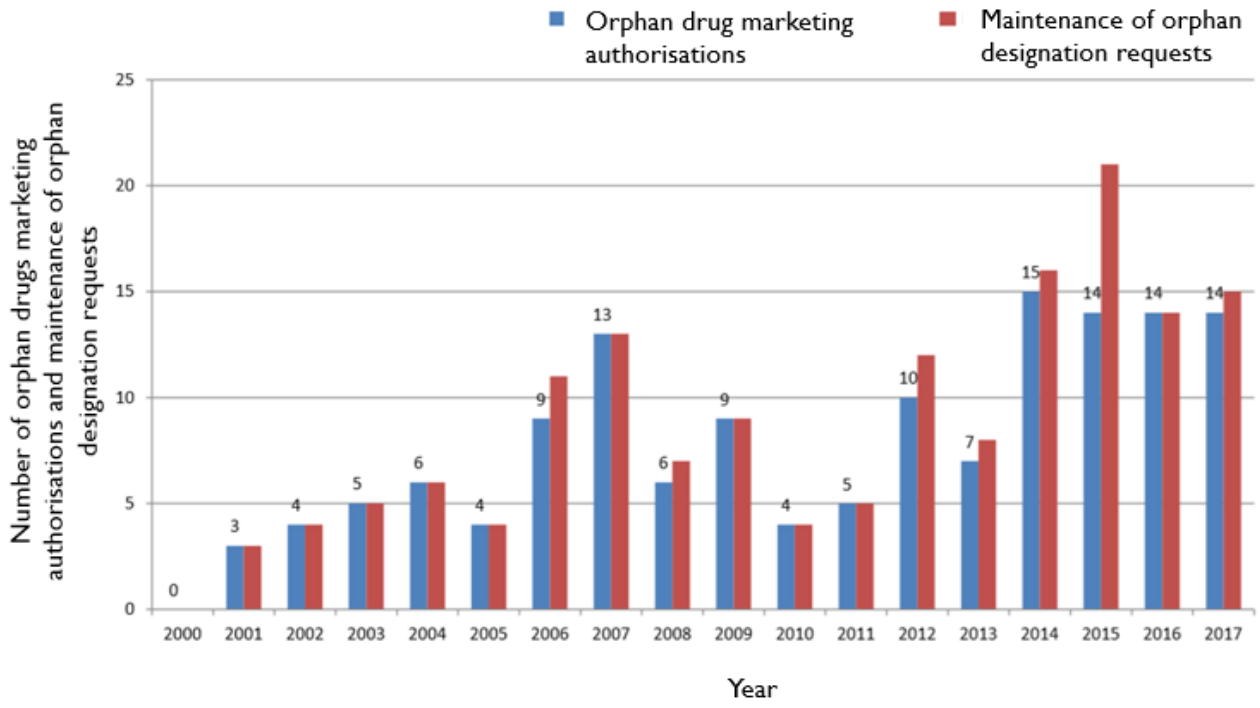

Figure 9: Orphan drug marketing authorisations and maintenance of orphan drug designations requests provided in European Union, since 2000 until 2017. Reproduced from source [127].

In this context, the applicant should request orphan drug designation soon enough to benefit from all the incentives but using data that is consistent and solid enough to support the success of the orphan drug designation request and, thereafter, the orphan drug marketing authorisation for that therapeutic indication. Both clinical and preclinical data, in a valid *in vivo* model and/or preliminary clinical data, are accepted for supporting the potential orphan drug designation. Most of the applications that had a positive opinion by the COMP and obtained orphan designation by the EC were based on clinical data (Fig. 10a). Nevertheless, a considerable number of applications comprise only preclinical data (Fig. 10b) [5].

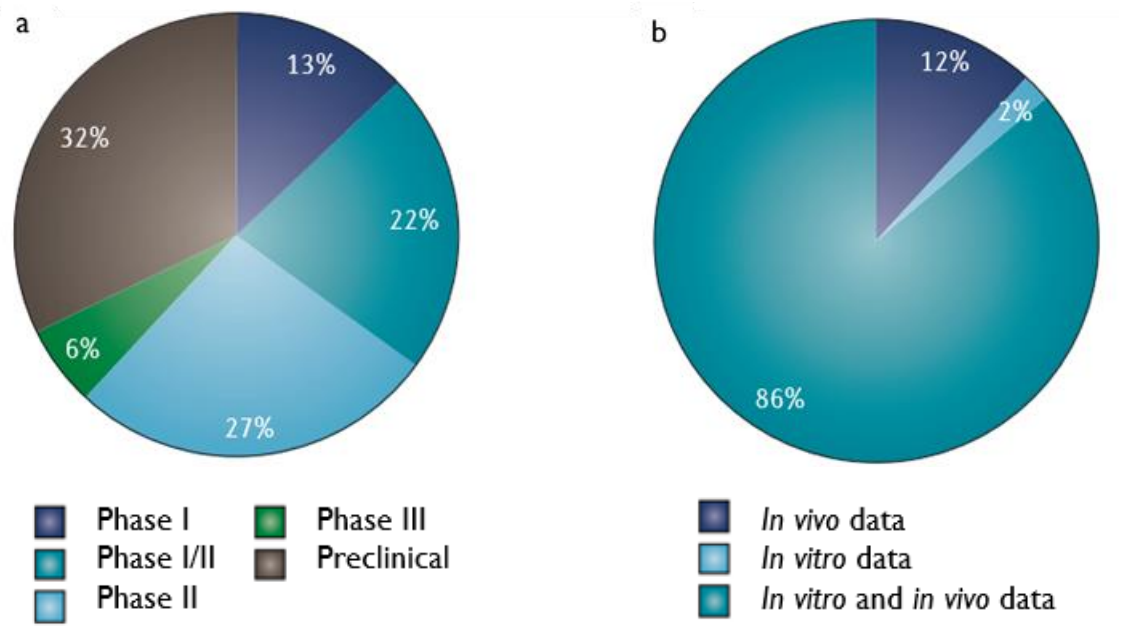

Figure 10: Applications for orphan drugs designations, for which a positive opinion by COMP was issued and orphan designation was granted by the EC, from 2000-2014:
a| The development phase of the products for which orphan drugs designation was submitted.
b| The level of preclinical evidence in applications submitted. Reproduced from source [5].

At the end of a successful clinical development process and, if orphan drug designation is maintained and marketing authorisation is given, the marketing authorisation holder can benefit of the 10 years of market exclusivity for the orphan indication concerned [118]. Also, it is necessary to ensure that there are no other orphan medicines authorised in the market, similar to the new medicine. Therefore, the applicant should attach a similarity report. A medicine is considered similar to other depending on its principal structural features, mechanism of action and therapeutic indication [118]. However, for the same therapeutic indication, a medicine may break marketing exclusivity under the conditions referred previously.

6. ORPHAN DRUGS MARKET

6.1. MARKET ANALYSIS: ADVANTAGES AND OPPORTUNITIES

The declining pipelines of new therapies have led to an increasingly challenging competition within the pharmaceutical industry. Therefore, pharmaceutical companies are diversifying their portfolios and exploring new disease areas and pathways to enhance pipeline value, including the targeting of rare diseases [122]. The incentives for orphan drug research and development have been stimulating pharmaceutical sponsors to improve the health of patients with rare diseases worldwide [122].

Looking at the EUA case, only 10 treatments for rare diseases were approved by the FDA and brought to the market in the 10 years before the Orphan Drug Act enactment [123]. In the last few years, orphan drug designation requests increased so much that the FDA needed to restructure their framework for orphan drug approval processes (Fig. 11) [124] [125]. In June of 2017, FDA put into action a modernization plan whose main goals were to eliminate the backlog of existing orphan designation requests and make sure that new applications were always answered on time. For that, the FDA brought together a team of senior experts including reviewers and created a new template to facilitate reviews of the new designation requests [126].

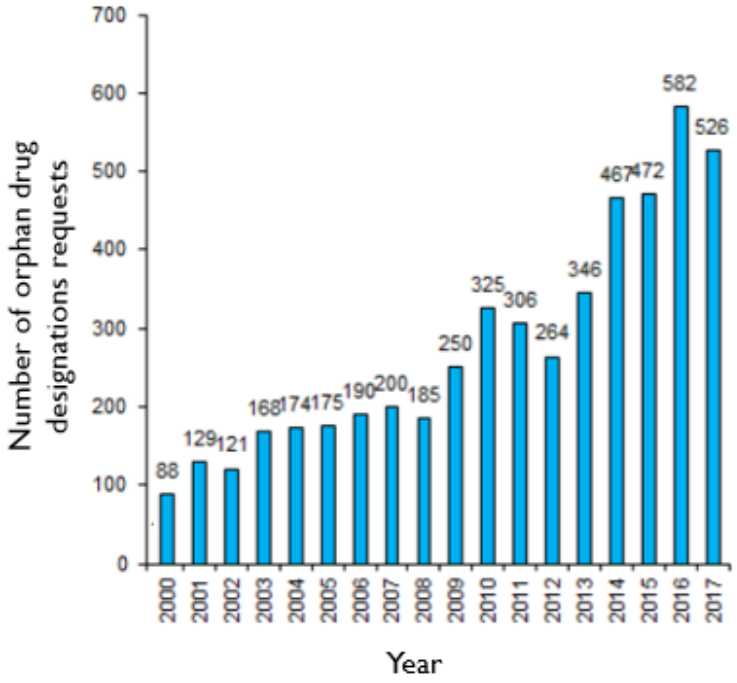

Figure 11: Number of FDA orphan drug designations requests from 2000 to 2017. Reproduced from source [125].

Likewise, the investment in orphan drug medicines in the EU increased abruptly in the last years. Since the year 2000, more than 2974 applications for orphan drug designation were submitted and the COMP has emitted 1971 positive opinions and 26 negative opinions. There were 784 withdrawals during the assessment mainly due mainly to safety and efficacy concerns. In total, over 1952 orphan designations have been issued by the EC (Fig. 12) [127]. It is important to highlight that almost all positive opinions emitted by COMP are followed by an orphan designation issued by the EC.

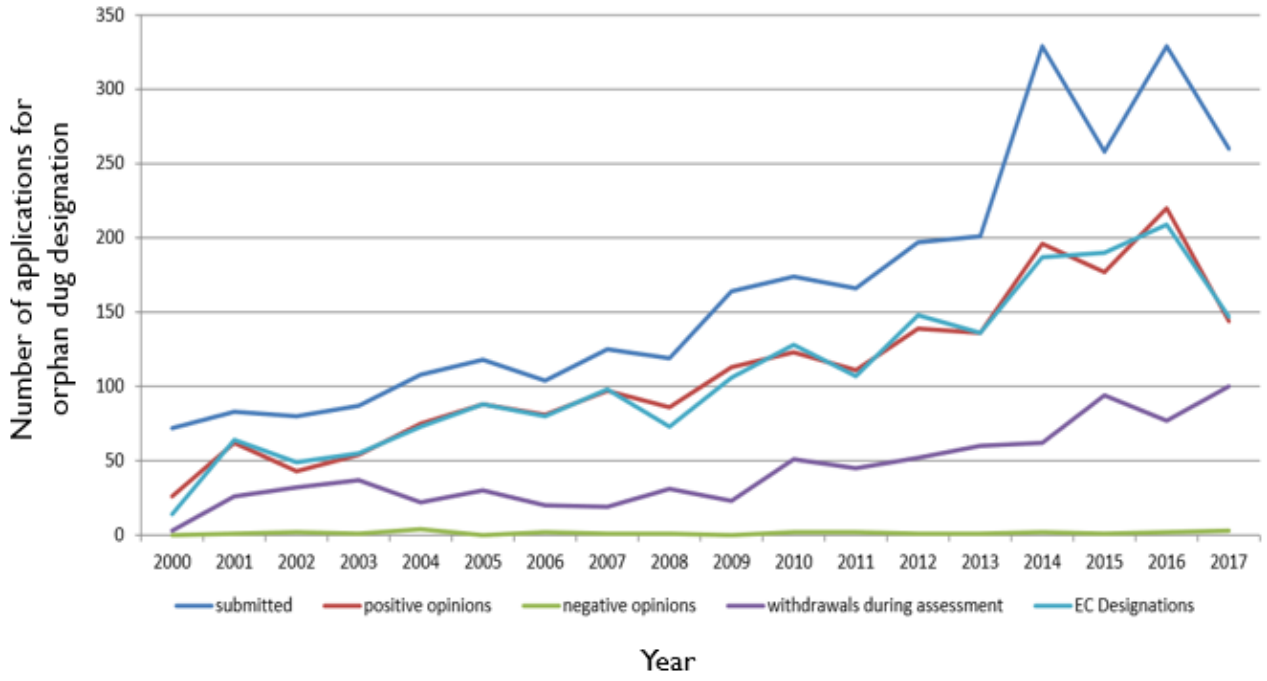

Figure 12: Number of applications for orphan drug designation sent to the EMA from 2000 to 2017. Reproduced from source [127].

The increase in the development of orphan drugs demonstrates the need for pharmaceutical companies to restructure the organization in their Research and Development departments and change their approach to be more effective. Furthermore, investing in the orphan drugs field could help overcome the lack of innovation in the area of drug discovery [20].

In this context, there are some factors that made orphan drugs an attractive investment, such as: possibility of shorter timelines, more flexibility and support from the regulatory authorities and financial incentives [121] [128].

This period of growth in the number of orphan drug approvals reflects also the industry focus on developing targeted therapies and supporting the evolution to a stratified

and personalized medicine [122]. A possible explanation for this success rate is the genetic evidence that supports drug development and guides target selection [21].

Overall, these considerations have turned orphan drug development strategies a hot topic for pharmaceutical companies and venture capital market, as well as to biotechnology enterprisers [128].

6.2. MARKET STRATEGIES: THE ROLE OF SMALL BIOPHARMACEUTICAL COMPANIES

The regulatory support given to orphan drug development provided an increase in the number of new clinical trials for rare diseases, and an incentive in developing innovative and personalized drug technologies as well as identifying new rare diseases [20]. Small biopharmaceutical companies have seen their importance increase within this field, being innovation drivers for drug development [129]. These companies usually have a lower clinical development success rate comparing to big pharmaceutical companies due to their limited resources and internal experience, so generally they invest in small development programs for very specific targets. Often, they are pioneers in testing new therapeutic approaches in order to be competitive, so they focus their effort on niche markets such as rare diseases research [129]. Moreover, the research focus on rare diseases allows small companies to overcome certain difficulties caused by their limited funding due to the incentives that are given, even if such diseases present their own challenges. Examples of small biopharmaceutical companies that achieve regulatory success for some orphan drugs designated are presented in Table 8 [129].

One of the strategies that small companies use to benefit from the investment in this field is sharing their specialized scientific knowledge and know-how with big pharmaceutical companies. Then, these big companies can acquire orphan drug candidates or even the entire small company to complement their internal projects. This way, the potential of small biopharmaceutical to generate value is recognized by bigger companies, consequently, increasing their market value as innovation drivers [20].

Other strategy used by small biopharmaceutical companies to achieve success in the pharmaceutical field is developing new formulations with already known active substances or improving their security or efficacy profiles. Therefore, this type of companies can bring innovative ideas to optimizing manufacturing processes and methods [129].

In the last few years, many of the new drug approvals were for orphan indications, which evidences the importance of research in this field [20]. The inclusion of these small companies as well as academic projects in drug market pushes for changes within the Research and Development strategies of big pharmaceutical companies, fostering innovation in the drug discovery process.

Table 8: Examples of small biopharmaceutical companies that had regulatory approval by FDA between 2014 and 2015, using orphan designation as strategic approach. Reproduced from source [129].

Pharmaceutical Company	Orphan Drug	Therapeutic Indication	Other information
Retrophin®	Cholic Acid (Cholbam®)	Bile acid synthesis disorders	-
Synageva BioPharma	Sebelipase alfa (Kanuma®)	Lysosomal acid lipase deficiency	Acquired by Alexion in 2015
Wellstat Therapeutics Corporation	Uridine triacetate (Xuriden®)	Hereditary orotic aciduria	-
topotarget	Belinostat (Beleodaq®)	Peripheral T-cell lymphoma	Merged with BioAlliance and become Onxeo
BIOMARIN®	Elusulfase alfa (Vimizin®)	Mucopolysaccharidosis IV type a	-
VANDA PHARMACEUTICALS INC.	Tasimelteon (Hetlioz®)	Sleep-wake disorder in blindness	-
InterMune®	Pirfenidone (Esbriet®)	Idiopathic pulmonary fibrosis	Acquired by Roche in 2014

6.3. ORPHAN DRUGS PRICING

For a long time, rare diseases were seen as an unattractive investment due to the high cost of drug development process that could difficultly be recovered due to the small populations affected [130]. This idea has changed with the implementation of laws and incentives afforded by regulatory authorities to promote orphan drugs development. Market analysis suggests that the impact of treating a smaller population is compensated by several facts: higher pricing of orphan drugs, longer exclusivity period and faster reimbursement rate due to the unmet medical needs in most of rare diseases as well as other factors [122].

Furthermore, there is evidence that time spent on clinical trials is shorter and the regulatory process has a higher success rate for orphan drugs than for non-orphan-drugs (Fig. 13). Therefore, revenue potential may be the same for orphan drugs as for non-orphan drugs, even with a significantly reduced population [122].

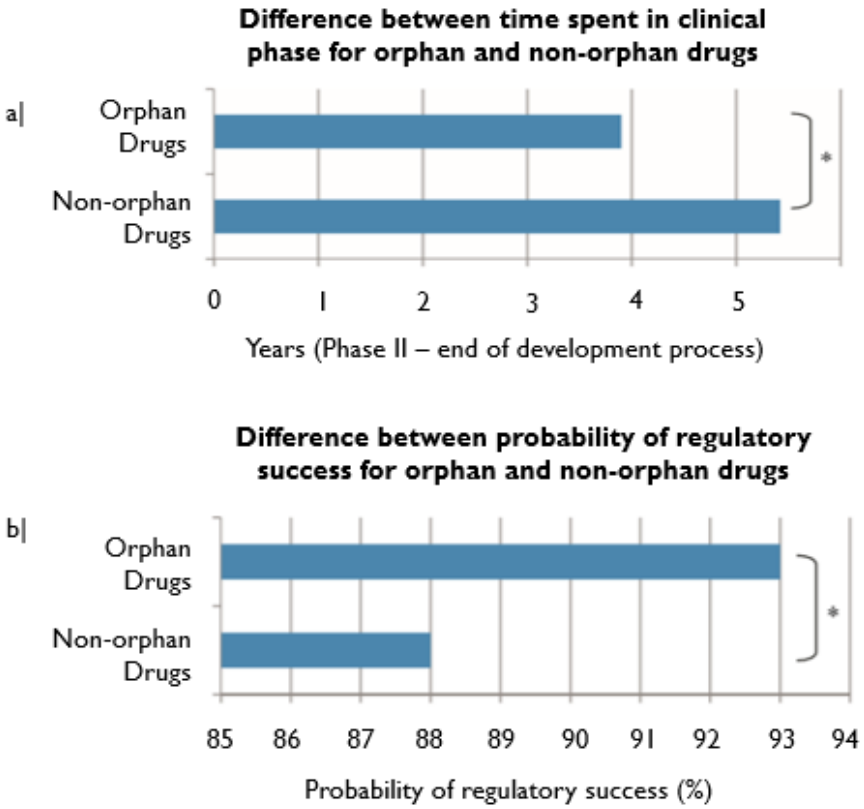

Figure 13: Orphan drugs development process present:

a| decreased clinical trial development times since the beginning of phase II until the end of orphan drug development process

b| greater probability of regulatory success compared with non-orphan drugs. * $p<0.05$.

Reproduced from source [122].

As the probability of regulatory success is higher for orphan drugs when compared to non-orphan drugs, some drugs which had initially been approved as an orphan drug, have then been granted authorisations for other indications, as it is more likely for a drug that was developed for an orphan indication to achieve regulatory success [122]. For instance, infliximab (Remicade®) had its first approval by the FDA under orphan drug designation for treating a certain Chron disease population. Nowadays, it is used for several other therapeutic indications including non-orphan indications such as rheumatoid arthritis [131] [132].

Commercial potential for some orphan drugs still need some adjustments in order to be adapted to the health care system [99]. In order to reach an economically viable solution there is a need to find a balance between the unmet needs of the patients and research and production costs [122]. Approaches such as distribution of the payment over time and according to the improvement of the patient health status could be considered. For that, the therapy outcomes should be considered in order to adjust the price according to patient benefits [108].

Furthermore, to improve the current system of rare diseases research and make it cost-effective it is necessary to develop a profitable development process. For that, an open collaboration would be necessary to bring together the knowledge of the experts to work on optimization of rare diseases therapeutic approaches [10]. This exchange of information could benefit rare disease patients and boost scientific advances in this field, while reducing costs [10].

7. CONCLUSIONS AND PROPOSALS FOR THE FUTURE

Rare diseases and orphan drugs are currently a topic of research that draws a significant amount of interest from the scientific community. Despite differences in the definition of rare disease among countries, there is a global desire for collaboration between stakeholders involved in orphan drug development process. Success depends on cooperation between academic researchers, pharmaceutical companies, regulatory authorities, clinical research teams and patient's organizations [6]. Patient's organizations have an especially important role in this discussion as they can push international authorities to invest more in rare disease research and to face translational barriers. For example, the National Organization for Rare Diseases (NORD) has been an active voice in advocating for improvements to the orphan drug development process, through organisms developed for rare diseases patients support [133] [7]. Furthermore, this organization is a crucial communication link that provides patient information for clinical trials recruitment [32]. When involved in the early stage of the protocol development process, patient's organizations can help to define not only the most relevant clinical endpoints, but also the most appropriate centers, and disseminate information about the clinical study to patients, according to local regulations [134].

Before reaching the clinical stage, the development process of orphan drugs faces difficulties in the preclinical phase already. The main challenge for scientists who are working on orphan drugs discovery and development is the identification of possible targets. Thus, there is a need for investments in better understanding the pathophysiology of rare diseases. Likewise, preclinical studies need to present appropriate disease models to perform the tests required to validate the orphan drug's safety and efficacy and to be translated into human-focused research.

Regarding their clinical development process, orphan drugs face distinct challenges when compared to non-orphan drugs. Clinical trials for orphan drugs tend to have a limited number of patients, a heterogeneous and dispersed population, and they don't follow the well accepted RCT design [135]. One of the main hurdles is working with the limited evidence obtained from clinical trials in small populations [26]. This could be tackled using alternative and adaptive clinical trial designs. They represent an opportunity to shorten the time spent in the clinical development process for orphan drugs, without compromising their validity. Besides that, these trials can recognize an ineffective treatment at an earlier stage when compared to RCTs, which makes them more successful.

Other clinical phase hindrances can be overcome using biomarkers as they allow the quantification of biologic characteristics that can define a diagnosis and monitoring disease progression. Biomarkers are an accurate evaluation tool with several applications in the clinical drug development such as the definition of clinical endpoints [37]. Apart from that, new progresses in genetics and biotechnology provide a better clinical development strategy such as genetic-based selection of clinical trial subjects, as well as hope in future therapeutics that have already reached this phase.

Lack of therapeutic solutions for rare diseases creates an excellent opportunity for the development of new treatments for diverse therapeutic areas. New therapeutic approaches for rare diseases usually involve targeting and application of personalized medicine which allows clinical researchers to learn with each of the patients' disorders and their specificities, even within subsets of the same disease [12]. Gene therapy is probably the most disruptive area for clinical research that can bring benefits to patients with rare diseases within the next few years [32].

To stimulate research on orphan drugs, regulatory authorities provide incentives that facilitate this process, while providing scientific support. The increase in the number of approvals of orphan drugs in recent years proves that the efforts made by stakeholders involved in the orphan drug development process are working. Furthermore, market evolution for this sector is favourable. Pharmaceutical companies are investing in rare diseases research and are interested in including orphan drugs in their pipeline.

However, adjustments need to be done in health care systems to make orphan drugs available to all patients with rare diseases. The rareness, diversity and severity of these diseases make them a public health problem difficult to deal with. However, some achievements in the last years have brought some changes to this scenario. Research funding programmes for rare diseases and commitment from the scientific community to develop new therapeutic approaches give the rare disease community hope in the discovery of breakthrough treatments.

Overall, the future seems to hold promising solutions that can significantly improve the lives of patients with rare diseases.

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