Universidade de Lisboa

Faculdade de Farmácia

Pharmaceutical Regulation of Nanomedicines: Principles and Challenges

Inês Rodrigues Lousa

Monography supervised by Full Professor Helena Isabel Fialho Florindo Roque Ferreira and co-supervised by Full Professor António José Leitão das Neves Almeida.

Master's in Pharmaceutical Engineering

2024

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Final Work for the Master's Degree in Pharmaceutical Engineering presented to the Universidade de Lisboa through the Faculdade de Farmácia

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Resumo

O termo nanotecnologia foi introduzido em 1959 pelo físico Nobel Richard Feynman, na sequência de uma apresentação intitulada "Há muito espaço no fundo". A introdução da nanotecnologia no desenvolvimento de produtos farmacêuticos para uso em humanos requer a adaptação dos quadros regulamentares existentes às propriedades exigentes dos nanomateriais, bem como ao impacto que essas propriedades apresentam no que diz respeito às alterações induzidas nas funções das células.

No presente trabalho foi efetuada uma avaliação dos atuais procedimentos regulamentares para a análise dos pedidos de introdução no mercado de novos medicamentos na União Europeia e nos Estados Unidos. Realizou-se uma profunda revisão da literatura sobre os esforços que a EMA e a FDA têm feito ao longo dos anos para estabelecer diretrizes específicas para os nanomedicamentos.

Com uma compreensão da regulamentação e das estratégias que estão a ser implementadas para a adaptar aos nanomedicamentos, procedeu-se à identificação de fatores que impedem o sucesso das translações clínicas. Verificou-se uma forte correlação entre a falta de diretrizes regulamentares e as dificuldades que os fabricantes encontram ao longo do processo de produção, existindo uma lacuna significativa de informação de qualidade e segurança dos nanomedicamentos, particularmente de protocolos normalizados e de CQAs. Dada esta incerteza e a carência de critérios para os fabricantes, é frequente que os nanomedicamentos terminem os ensaios clínicos com grandes margens de fracasso.

Concluiu-se que tanto a EMA como a FDA têm sido cautelosas na avaliação dos nanomedicamentos e que a análise caso a caso, com base na regulamentação extensa, sólida e eficaz em vigor, é a escolha certa. Além disso, as vastas iniciativas lançadas para desenvolver orientações que cubram a falta de dados mencionada são essenciais, e a mudança para um papel mais proactivo dos reguladores é vital, com um envolvimento prematuro na fase de desenvolvimento.

Palavras-chave: nanomedicamentos, regulamentação, translação clínica, EMA, FDA**.**

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Abstract

The term nanotechnology was introduced in 1959 by Nobel physicist Richard Feynman, following a presentation entitled "There's plenty of room at the bottom". The introduction of nanotechnology in the development of pharmaceutical products for use in humans requires the adaptation of existing regulatory frameworks to the demanding properties of nanomaterials, as well as the impact that these properties have in terms of the changes induced in the functions of cells.

This study evaluated the current regulatory procedures for analysing marketing applications for new drugs in the European Union and the United States. An in-depth literature review was carried out on the efforts that the EMA and FDA have made over the years to establish specific guidelines for nanomedicines.

With an understanding of the regulations and the strategies that are being implemented to adapt them to nanomedicines, factors that hinder the success of clinical translations were identified. A strong correlation was found between the lack of regulatory guidelines and the difficulties that manufacturers encounter throughout the production process, and there is a significant gap in information on the quality and safety of nanomedicines, particularly standardised protocols and QCs. Given this uncertainty and the lack of criteria for manufacturers, nanomedicines often end clinical trials with large margins of failure.

It was concluded that both the EMA and the FDA have been cautious in evaluating nanomedicines and that analysing them on a case-by-case basis, based on the extensive, solid and effective regulations in force, is the right choice. In addition, the extensive initiatives launched to develop guidelines to cover the lack of data mentioned are essential, and the shift towards a more proactive role for regulators is vital, with early involvement in the development phase.

Keywords: nanomedicine, regulation, clinical translation, EMA, FDA.

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Declaro ter desenvolvido e elaborado o presente trabalho em consonância com o Código de Conduta e de Boas Práticas da Universidade de Lisboa. Mais concretamente, afirmo não ter incorrido em qualquer das variedades de fraude académica, que aqui declaro conhecer, e que atendi à exigida referenciação de frases, extratos, imagens e outras formas de trabalho intelectual, assumindo na íntegra as responsabilidades da autoria.

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Nomenclature

2D – Two-dimensional

3D - Three-dimensional

AB - Amphotericin B

ABCD - Amphotericin B Colloidal Dispersion

ABLC - Amphotericin B Lipid Complex

ACNPs - Antibody-conjugated Nanoparticles

ADCs - Antibody-drug Conjugates

ADME – Absorption, Distribution, Metabolism and Excretion

Ag – Silver

AIDS - Acquired Immune Deficiency Syndrome

ANDA - Abbreviated New Drug Application

API - Active Pharmaceutical Ingredients

ATMPs - Advanced Therapy Medicines

Au – Gold

BE – Bioequivalence

BsUFA - Biosimilar User Fee Amendments

CAFs - Cancer-associated Fibroblasts

CAT – Committee for Advanced Therapies

CDER - Centre for Drug Evaluation and Research

CHMP – Committee for Medicinal Products for Human Use

COMP – Committee for Orphan Medicinal Products

CORES - Collaborative Opportunities for Research Excellence in Science

CP – Centralized Procedure

CPP - Critical Process Parameters

CQA - Critical Quality Attributes

CTD - Common Technical Document

DNA – Deoxyribonucleic Acid

DoE - Design of Experiment

DSS - Decision Support System

EC - European Commission

ECHA - European Chemicals Agency

EHS - Health and Environmental Safety

EMA – European Medicines Agency

EPR - Enhanced Permeability and Retention

EPR - Enhanced Permeation Retention

ESMO - European Society of Medical Oncology

EU – European Union

EU-NCL - European Nano-Characterisation Laboratory

FDA – Food and Drug Administration

GD – Gadolinium

GMPs – Good Manufacturing Practices

HER2 – Human Epidermal Growth Factor Receptor 2

HIV - Human Immunodeficiency Virus

ICH – International Council for Harmonization

IFIs - Invasive Fungal Infections

INPs - Inorganic nanoparticles

IPR - Intellectual Property Rights

ISO - International Organization for Standardization

ITSs - Intelligent Testing Strategies

IV – Intravenous

IVIVC - *In-vitro In-vivo* Correlation

LAB - Liposomal Amphotericin B

MAA - Marketing Authorization Application

mAb - Monoclonal Antibody

MD - Medical Devices

MP - Medicinal Products

MPS - Mononuclear Phagocytic System

N&N - Nanotechnology and Nanomedicines

NBCDs - Non-biological Complex Drugs

NCL - Nanotechnology Characterization Laboratory

NDA – New Drug Application

NHP - Nanotechnology-enabled Health Product

NLCs - Nanostructured Lipid Carriers

NMs – Nanomaterials

NNI - National Nanotechnology Initiative

NNI - National Nanotechnology Initiative

NPs – Nanoparticles

NTA - Nanoparticle Tracking Analysis

NTF - Nanotechnology Task Force

OECD - Organization for Economic Co-operation and Development's

OOAC - Organ-on-a-chip

PAT - Process Analytical Technology

PD – Pharmacodynamic

PDCs - Polymer-drug conjugates

PDUFA - Prescription Drug User Fee Act

PK - Pharmacokinetic

PLM - Product Life-Cycle Management

PNPs - Polymeric nanoparticles

PRAC – Pharmacovigilance Risk Assessment Committee

PSD - Particle Size Distribution

QTPP - Quality Target Product Profile

R&D – Research and Development

REACH - Registration, Evaluation, Authorization and Restriction of Chemicals

RIPoN - Reach Implementation Project on Nanomaterials

RNA – Ribonucleic acid

RP - Regulation Preparedness

SbD - Safe-by-Design

SIA - Safe Innovation Approach

SLNs - Solid Lipid Nanoparticles

SmPC - Summary of Product Characteristic

SPOs - Standard Operating Procedures

STM - Scanning Tunnelling Microscope

TE - Designated Trusted Environments

TME - Tumour Microenvironment

US - United States

USA – United States of America

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1. Introduction

Nanotechnology as a term was introduced in 1959 by Nobel physicist Richard Feynman, as the result of a meeting of the American Physics Society entitled “There is plenty of room in the bottom.” (1) The talk encompassed the idea of physically manipulating materials at the atomic level. Nonetheless, the idea went somehow unnoticed and irrelevant until a few years later. In 1974, Nario Taniguchi described Nanotechnology as “the processing of separation, consolidation, and deformation of materials by one atom or one molecule”. Although Nanotechnology is often associated with the 21st century, the first evidence of the use of nanotechnology dates back to the ancient romans, with the use of colloidal gold (2). In 1981 there was an impressive breakthrough in technology that opened the possibilities of nanotechnology development with the invention of the Scanning Tunnelling Microscope (STM) at the IBM® Laboratory in Zurich (3), which allowed surface image reconstructions at the atomic scale, and the discovery of several novel nanomaterials and nanostructures with useful applications.

Serious investment and research work into nanoscience was noticeable in the beginning of the 21st century, with governments such as the United States of America (USA) backing nanotechnology development with significant funding programmes. In Figure 1 is visible the evolution of publications involving nanotechnology, from the year 2000 to 2022, including number of publications and annual growth rate (4).

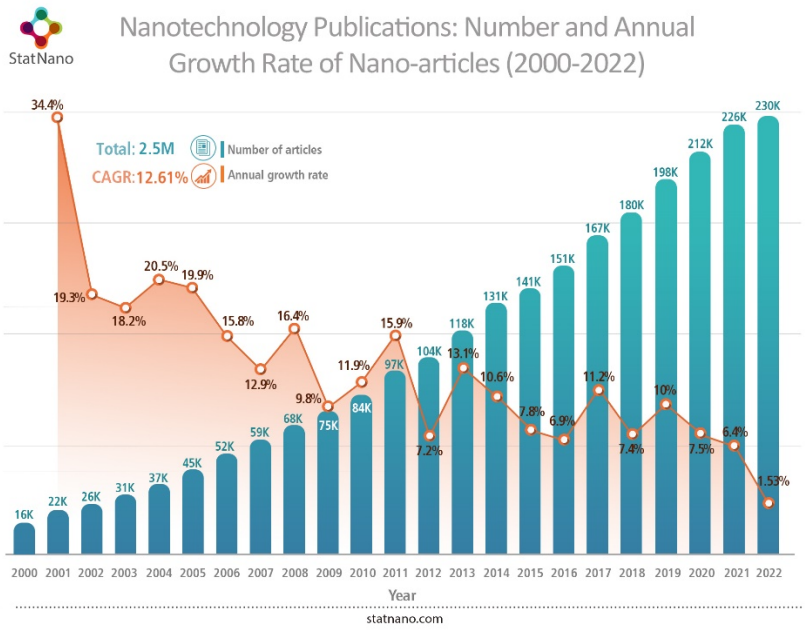

Figure 1- Annual number of publications involving nanotechnology and annual growth rate, from the year 2000 until 2022. Adapted from (4).

With increase in research a broad spectrum of applications started to appear in the most varied areas such as electrical engineering, material engineering, chemical applications or in the energy sector. Medicine and Healthcare are areas where nanotechnology has proven to be extremely helpful with numerous applications for diagnostics, therapies, and strategies combining both (theranostics). In this area, the goal is to exploit the unique physicochemical properties of nanomaterials to produce or enhance strategies, systems, or devices for the diagnostics and/or treatment of diseases (5). Nanomedicine is considered as one of the areas where nanomaterials can have a significant impact, however, being a heavily regulated sector, care must be taken to ensure that research initiatives translate into actual applications.

Generally, a nanomaterial is defined by having one of its characteristic dimensions, either internal or external, between 1 and 100 nm. However, measuring dimensions at this scale has proven complex and often misleading. Therefore, before advancing towards any kind of regulatory framework it is essential to establish concrete definitions for the classification of nanomaterials, more specifically nanomedicines, to clearly define which drug products are within the scope of such regulation.

For instance, the European Commission (EC) defines a nanomaterial as a "natural, incidental or manufactured consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50 % or more of these particles in the number-based size distribution fulfil at least one of the following conditions: (a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm; (b) the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm; (c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm". Legislation and policies applied in the European Union (EU) regarding nanomaterials follow this criterion (6).

In their latest document, the Food and Drug Administration (FDA) follows a different definition. The FDA considers a material possessing one of its characteristic dimensions in the nanoscale range (1 to 100nm) or any material exhibiting properties or phenomena solely attributed to their dimensions (up to 1000nm) to be included in the nanomaterial category (7).

Regarding the European definition there are three fundamental aspects to identify the presence of a nanomaterial. Size, particle size distribution (PSD) and surface area. When it comes to size, as previously mentioned, the usual limit is set at 100 nm, however, this value cannot be assumed as an absolute criterion. The definition of the nanoparticles (NPs) must prioritize the behaviour of the properties instead of

defining a static size range, more in tune with the American definition. The PSD is one of the most important attributes of nanomaterials and their characterization. It reflects the size distribution observable within the nanomaterial which often is polydisperse. PSD impacts product stability, in addition to leading to high variability in terms of biological responses (8). The surface area, in particular the surface area to volume ratio is also a parameter used to classify nanomaterials by the EC, with samples presenting values below $60 m^2/cm^3$ being encompassed in the nanomaterial category (6).

Nevertheless, it is important to clarify that despite the quantitative definition of 'nano' being clear, that does not apply to the point at which a particular material can be classified as a nanomaterial. The definition is as much based on dimension as it is on functionality, i.e., a material must be classified as a nanomaterial at the point it reveals a novel property resultant directly of its small size. This point differs among materials, hence, so does the aforementioned point.

There are two different approaches to manufacture nanoparticles, as represented in Figure 2, top down and bottom up. The former consists of the breakdown of a bulk material into a smaller size via mechanical or chemical processes, while the latter follows the inverse approach, starting with the atomic or molecular form and increasing in size. Both processes of manufacturing can result in different forms of particles, called primary particle, aggregate and agglomerate.

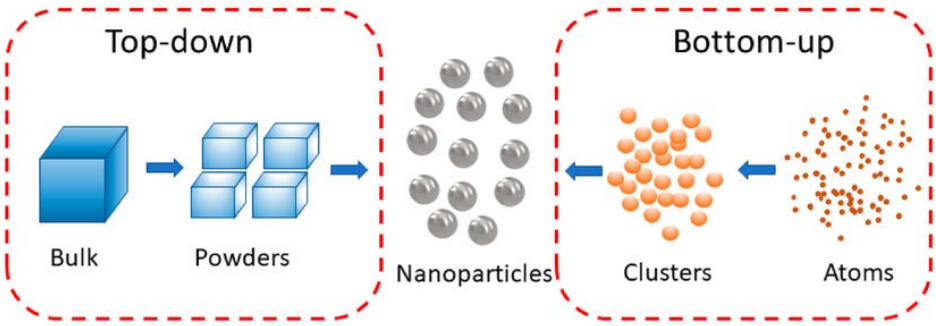

Figure 2- Top-down and Bottom-Up nanoparticle synthesis approaches. Adapted from (9).

Nanomedicines can be used for the treatment, diagnosis or prevention of several diseases. Usually, this type of medicines is formed by the combination of appropriate nanocarriers and active pharmaceutical ingredients (API), nonetheless, some of them can be prepared by transforming the API in a nano-sized component (e.g., nanocrystal) using stabilizers to avoid aggregation.

Some of the benefits of nanomaterials when compared to the traditional forms of the respective drugs are less toxicity, increased therapeutic effect and targeted delivery. This type of medicines can have a wide range of applications such as

bacterial, fungal, viral, and parasitic infections, cancer, blood diseases, immune diseases, ocular diseases, skin diseases and other indications (Figure 3) (10).

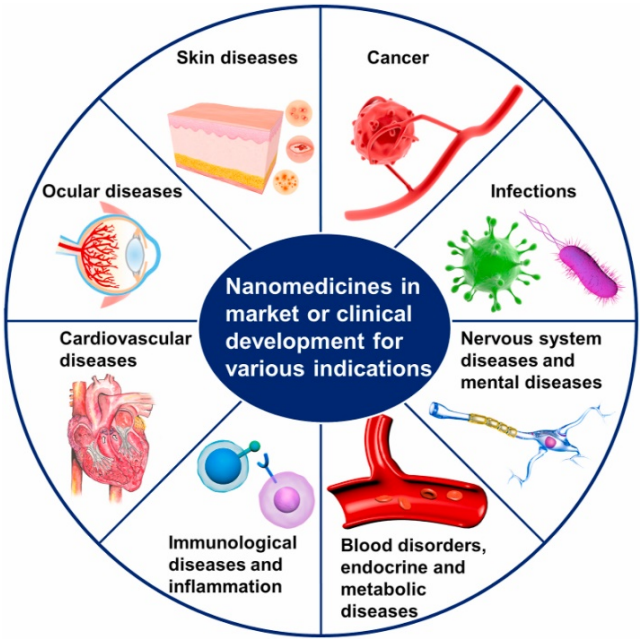

Figure 3- Nanomedicines in the market and various stages of clinical translations for many types of diseases. Adapted from (10).

As of July of 2022, there were 100 nanomedicines with granted access to the market, with an additional 563 drug products in clinical development or other manufacturing stages. Most of these nanomedicines are in clinical phase I (33%) and phase II (21%), being the majority focused on cancer (53%) and infection (14%) treatments. In addition to the use in the treatment of several diseases, nanomedicines can also be used in vaccine development and imaging diagnosis. The liposome or lipid-based nanoparticle is the most prevalent category of nanomedicines in the market or in clinical trials, with a share of 33%, followed with antibody-drug conjugate (15%), polymer-drug/protein conjugate (10%) and polymer (10%), as mentioned in Figure 4 (10).

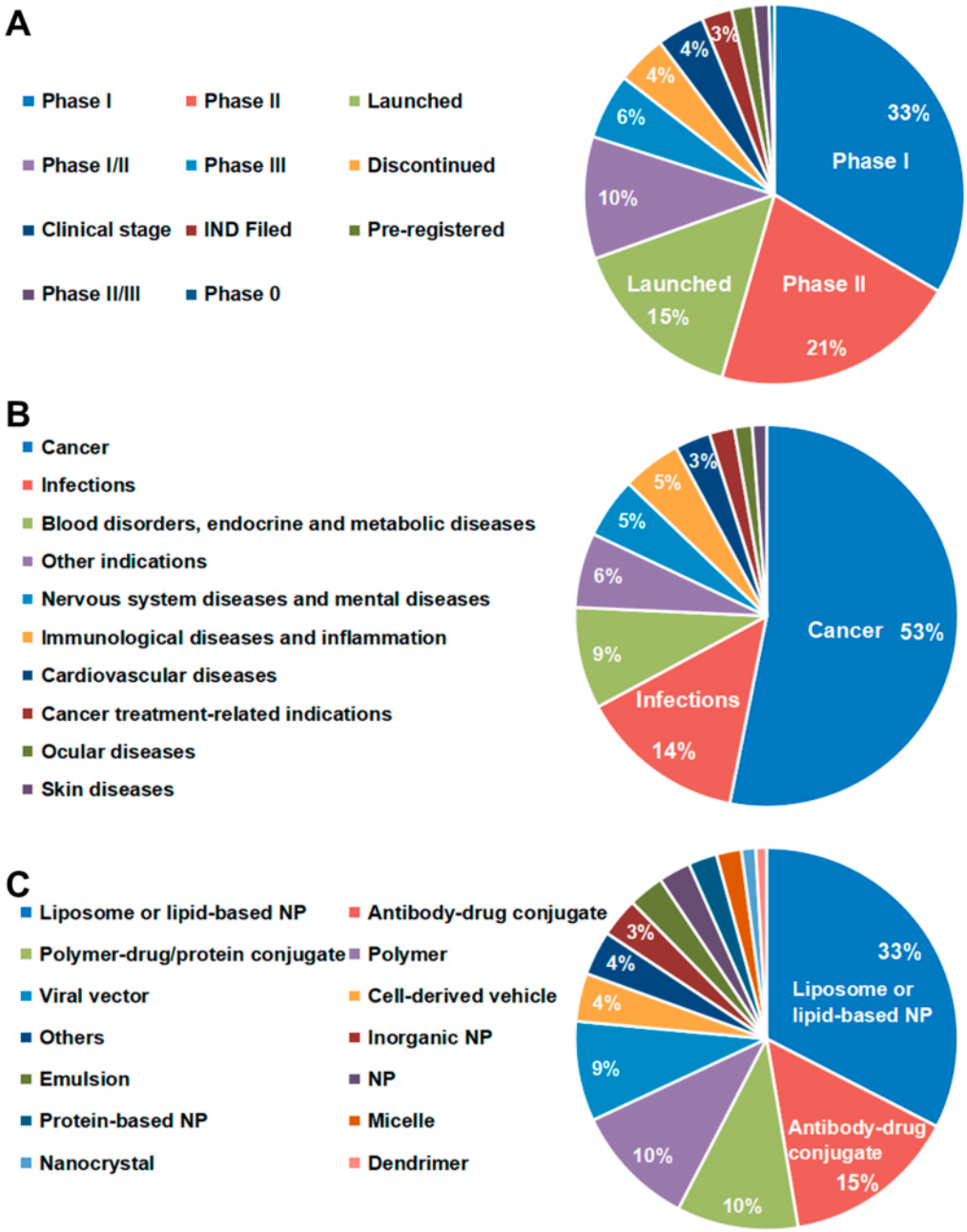

Figure 4- Overview of nanomedicines accessible in the market or in clinical translation. (A) Development status, (B) indications and (C) formulations. NP, nanoparticle. Adapted from (10).

One important nanomedicine that deserves special attention in particular are the approved COVID-19 vaccines containing lipid nanoparticles which posed a tremendous challenge from a regulatory standpoint due to the urgency in fulfilling the vaccination needs caused by the pandemic. Both EMA and the FDA had to adapt and create emergency committees and teams to expedite the regulation approval, analysing each vaccine on a case-by-case basis. Exceptions were created to allow rapid approval including earlier submission of the MA dossiers without all the data necessary in standard submissions, dossier rolling review along ongoing clinical

testing and shortening of the EMA/FDA standard deadlines (11). Large-scale manufacturing of the vaccine was also started while testing was being performed and with continuous scientific advice from regulatory agencies. The effort and experience brought by this crisis definitely created an opportunity to investigate into expedited forms of marketing authorization and a regulatory approach that develops alongside the manufacturing.

2. Classification of Nanomedicines

Regarding to the type and structure of the carriers, nanomedicines are generally classified as lipid nanosystems (liposomes, solid lipid nanoparticles, nanostructured lipid carriers, etc.), antibody-drug conjugates, inorganic nanoparticles, polymeric nanoparticles, dendrimers, micelles, polymer-drug conjugates, virus-derived vectors, nanocrystals, cell-derived carriers and protein-bound nanoparticles. The particular biological properties of nanomedicines derive from their nanoscale, specific structure, shape and surface characteristics such as hydrophobicity and surface charge. These features give them several advantages, including enhanced drug solubility and stability, higher drug selectivity, controllable drug release, synergistic delivery of multiple drugs, increased bioavailability, improved therapeutic effects and decreased adverse effects. The pharmacokinetic characteristics of nanomedicines are dependent on their formulations and, according to each design, advantages or drawbacks can arise.

2.1. Dendrimers

Dendrimers are tree-like branched structures that begin with an inner core and are developed with increasing concentric layers, usually labelled dendrons which evolve from generations (ramification points), as visible in Figure 5. The outermost layer is functionalized to serve specific purposes such as fixing drugs or targeting moieties. These characteristics have created substantial attention for the application of dendrimers in medicine as nanocarriers for traditional drugs, proteins, Deoxyribonucleic Acid/Ribonucleic acid (DNA/RNA) and, in some cases, as intrinsically active nanoscale drugs. A detailed review on the application of dendrimers in nanomedicine can be found in the work of Dias et al. (12).

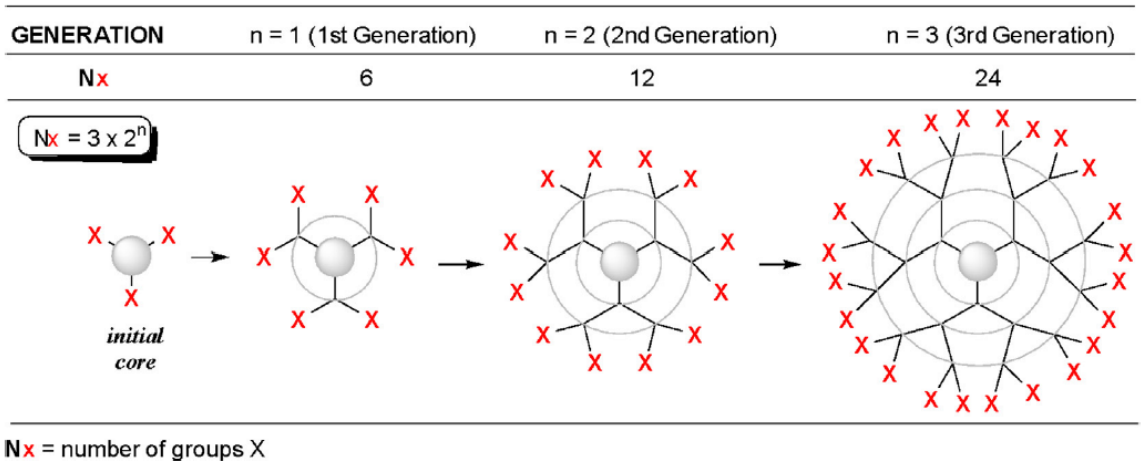

Figure 5- Generic dendrimer structure and stepwise synthesis process for dendrimers. Adapted from (12).

2.2. Lipid Nanosystems

Lipid nanoparticles have attracted attention in the field of nanomedicine particularly for the suitability to integrate drug delivery systems. Among many advantages, physical barriers such as the blood-brain interface can be easily surpassed by lipids without modifications and high protection of the active ingredients from degradative biological products can be warranted. These systems can be divided into two main categories, liposomes and lipid nanoparticles. Currently the most popular lipid nanosystems for drug carriers are solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) (13).

Liposomes are vesicular spheres composed of single or multiple phospholipid bilayers. According to size, number of bilayers and synthesis technique these are further subdivided. By encapsulating the active ingredient, liposomes can modify pharmacokinetic parameters and biodistribution, becoming effective drug carriers. SLNs are colloidal drug carriers that were first synthesized as an alternative to liposomes. The main drawbacks associated with SLNs are the low drug loading efficiency associated with their crystalline structure and the possibility of initial drug burst due to polymorphic changes in the solid lipid structure during storage (14). A general schematic representation of a SLN is presented in Figure 6.

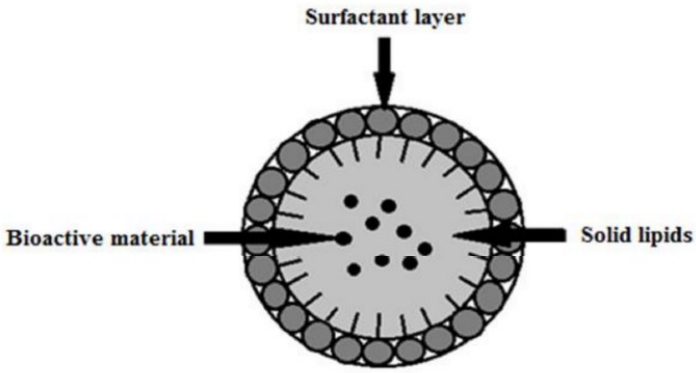

Figure 6- General structure of a solid lipid nanoparticle stabilized with a surfactant layer. Adapted from (15).

Nanostructured lipid carriers are a second generation of lipid nanosystems and were designed to overcome some of the SLNs limitations. NLCs usually include liquid lipid NPs that alter the lipid matrix to display better drug loading capabilities and physicochemical stability. More information on the synthesis and applications of lipid nanosystems in nanomedicine can be consulted in (9,11).

2.3. Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) can be synthesized from natural or synthetic materials and are employed in nanomedicines due to their simple elaboration, good biocompatibility, versatility, and ability to provide targeted delivery of drugs. PNPs have been proposed as ideal candidates for vaccines delivery, cancer therapies, and targeted antibiotics (16). Also, with appropriate choice of polymer the drug release rate can be highly controllable. They are mainly divided into two categories: nanocapsules and nanospheres, visible in Figure 7. At one hand, nanocapsules, because of their vesicular structures, hold the API in an aqueous or non-aqueous liquid core (located in the vesicle cavity) which is confined by the solidified polymeric shell. On the other hand, nanospheres can be interpreted as a solid of matrix polymers with the therapeutic substance uniformly dispersed throughout the particle. These do not have a core-shell structure, and drug release is achieved by diffusion or degradation of the polymer matrix. For further synthesis procedures, common materials, and applications the review of El-Say et al. (17) can be consulted.

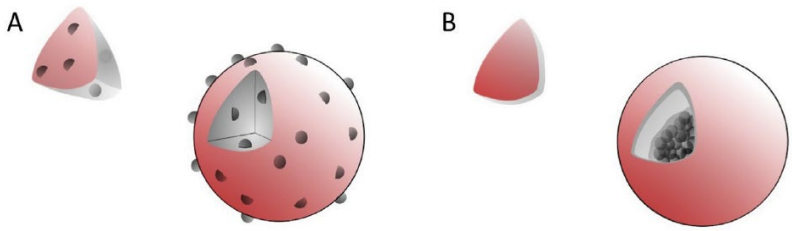

Figure 7- Different types of PNPs. A: Nanosphere (Adsorbed or Surface entrapped drugs); B: Nanocapsule. Adapted from (17).

2.4. Polymer-Drug Conjugates

Polymer-drug conjugates (PDCs) differ from polymer nanoparticles because no encapsulation or incorporation is involved. In PDCs the pharmaceutical active compound is covalently linked to a polymeric chain or backbone. The constituent parts of a PDC are the polymeric backbone, linker, targeting ligand, and drug substance (18). The linker is a fundamental part of PDCs in controlling the release mechanism of the drug substance under specific circumstances, the percentage of drug loading and drug stability. Usual synthesis techniques involve coupling reactions using coupling agents, polymerization procedures or, most recently, grafting procedures. One of the most common approaches is PEGylation, which accounts for the majority of approved PDCs. Another important category is the Polymer-Protein conjugates, in which the drug component is linked to a polymer backbone mimicking the structure or function of a protein. Pharmacokinetic parameters and drug release characteristics can be enhanced with this method. More information of PDCs is available in (18)(19)(20).

2.5. Antibody-Conjugated Nanoparticles

Antibody-drug conjugates (ADCs) are actively targeted nanomedicines composed of a monoclonal antibody (mAb) and a cytotoxic drug linked via blood-stable linkers. Using these components ADC take advantage of the specificity of mAbs, which are engineered to recognize a specific antigen, and the therapeutical ability of potent cytotoxic drugs. The cytotoxic drugs, also known as the payload, exert their effect after being released from the linker once the mAb has recognized the specific target. These compounds are mostly applied in cancer therapies and are extremely helpful in increasing therapeutical effects while reducing off cell toxicity (21). Antibody-conjugated nanoparticles (ACNPs) are similar to ADCs in the way they benefit from the mAb specificity to deliver targeted medicines. These nanoparticles are loaded with active substances, and the surface is functionalized with mAbs to recognize and bind to targeted antigens via chemical processes. The main advantages of ACNPs versus

ADCs is summarized in Figure 8 and the action mechanism of ACNPs is represented in Figure 9.

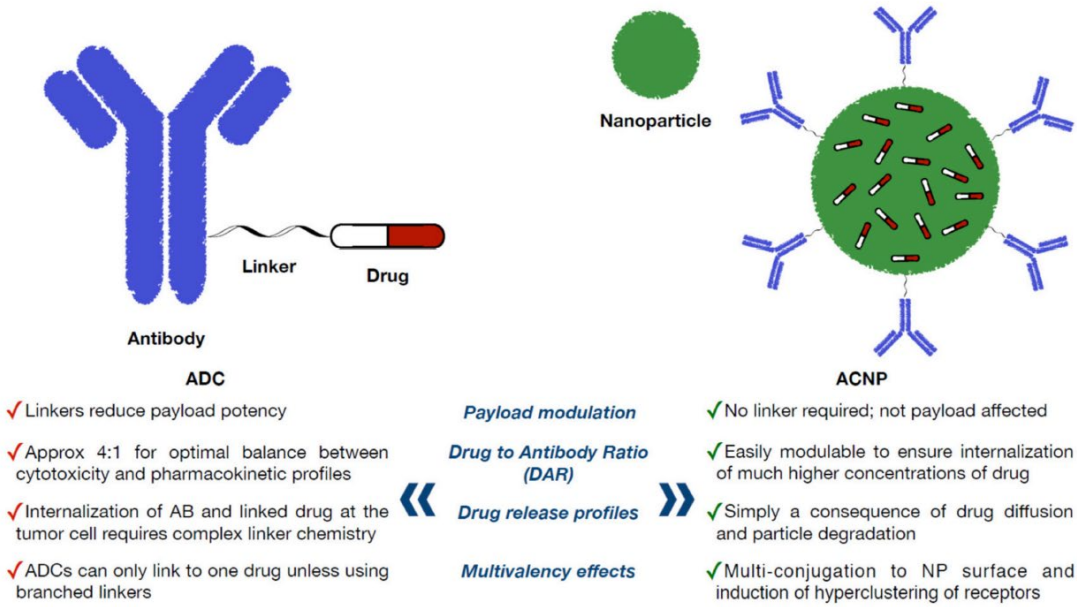

Figure 8- Comparison between ADCs and ACNPs. Adapted from (21).

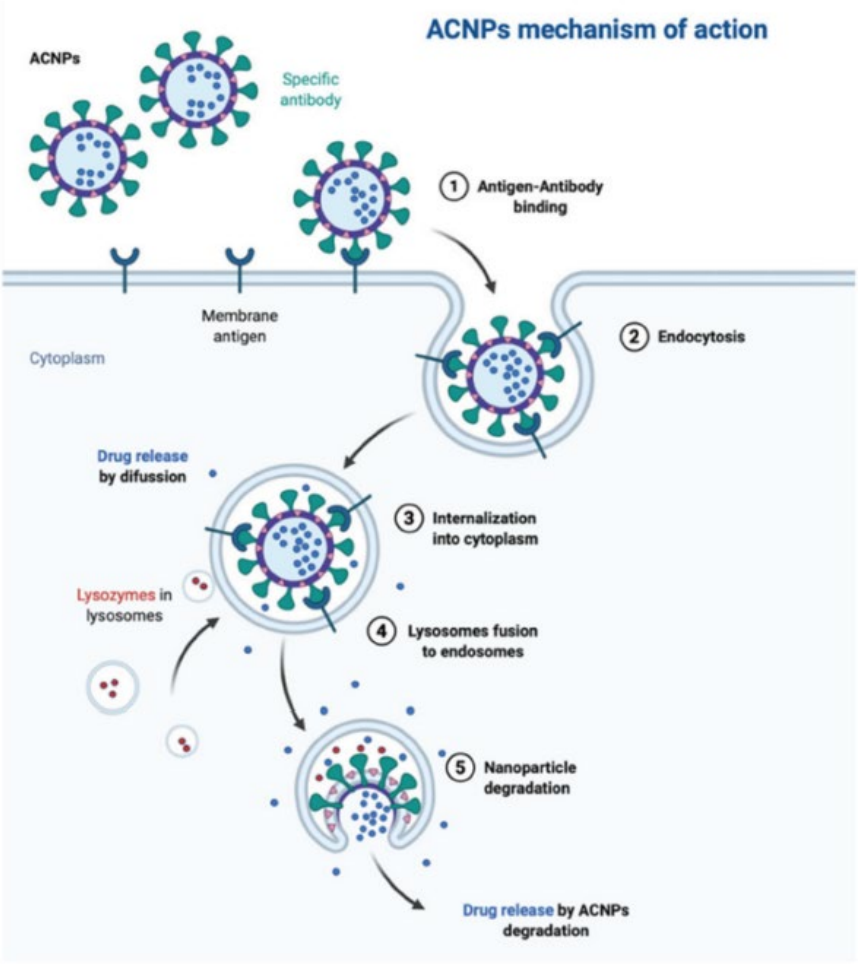

Figure 9- Action mechanism of ACNPs. Adapted from (21).

Concerning ACNPs there is still a significant lack of knowledge about the interaction between nanocarriers and biological systems, reported poor tumour accumulation, inadequate pharmacokinetics and a significant risk regarding the safety issue of raw materials for generation of NPs. Therefore, a higher failure rate in phase II and III clinical trials has been observed (22).

2.6. Nanocrystals

Nanocrystals are crystal nanoparticles of drug formulations, i.e., only composed of API stabilized or surrounding by a thin coating of surfactant without any carrier component as in the categories presented so far. According to the method of manufacture, bottom-up or top-down technology, they can be crystalline or amorphous. After nanonization, drug nanocrystals are usually conveyed into standard dosage forms, such as tablets, capsules or suspensions for intravenous (IV) administration. Hence, for regulatory purposes nanocrystals are considered a strategy to improve the standard formulations as opposed to a nanomedicine. Some of the advantages of the

nanocrystals are highly improved reproducibility and applicability to an extensive range of drugs with different solubility profiles. The surface chemistry of these nanoparticles can be altered to improve their stability, biocompatibility and targeting properties. Because to their minor size, they can penetrate deep into tissues and accumulate at disease sites, enabling more effective drug delivery. One of the weaknesses of nanocrystals is their potential toxicity, with several reports indicating cytotoxicity or inflammation *in-vitro* and *in-vivo*. Nevertheless, researchers are working to mitigate these risks by optimizing their size, surface chemistry, and surface coatings (23)(24).

2.7. Inorganic Nanoparticles

Inorganic nanoparticles (INPs) comprise metallic nanoparticles, such as gold (Au) and Silver (Ag), metal oxides, semiconductor materials and calcium phosphates. The most interesting application of these specific particles is in theranostics, i.e., the combination of diagnostics with therapeutics, a combination hard to achieve through lipid based nanosystems or dendrimers. Inorganic nanoparticles are attractive due to their ease of synthesis, low toxicity, extreme tunability and functionalization (25). As therapeutic agents, iron nanoparticles, gold nanoparticles and Hafnium-based nanoparticles have shown capabilities with some products receiving approval from the FDA and European Medicines Agency (EMA) mostly in cancer therapeutics (26). For imaging applications carbon-based inorganic nanoparticles and semi-conductor based nanoparticles, such as quantum dots, have been employed in imaging applications with success. Regarding theranostics applications with clinical success, gadolinium (GD) -based nanoparticles have been the leading prospects.

Despite the recognized potential and opportunities for inorganic NPs there are still hindering factors and challenges limiting their transition towards clinical development and implementation. Biological challenges such as the rapid clearance by the Mononuclear Phagocytic System (MPS), lack of biological barrier permeation capacity and toxicity from healthy tissue accumulation have slowed clinical progress of inorganic NPs. Technological challenges regarding safety, design, regulation and economics have also been reported. More information on the clinical development of inorganic NPs can be consulted in the work of Huang et al. (27).

2.8. Viral-Vectors

Viruses are molecules which have, over the years, developed a natural capacity to deliver their cargo of genetic material in specific targets and cells. This behaviour, if replicated, is the fundamental purpose of novel drug carriers and has led to significant interest in the development of virus nanoparticles and virus-like nanoparticles, the

latter not containing the genome part. Viral vectors are being studied for multiple applications with targeted drug delivery and gene therapy being the most noticeable. More information on viral vectors can be consulted in (28)(29).

2.9. Cell-derived Vehicles

In the field of nanocarriers and smart drug delivery agents, another important category are the cell-derived vehicles or drug carriers. Based on biological cells, such as immune or stem cells, such systems exhibit extended circulation, enhanced flexibility, low immunogenicity, and cytotoxicity with unique cellular assets. In addition, they are intrinsically biodegradable and biocompatible. Several therapeutics containing cell-based delivery systems have been approved (30). In Figure 8 a brief description of cell-based vehicles is presented.

Cell derived nanomedicines focus on using nanoscale materials that are derived from cells to enhance drug transport and delivery.

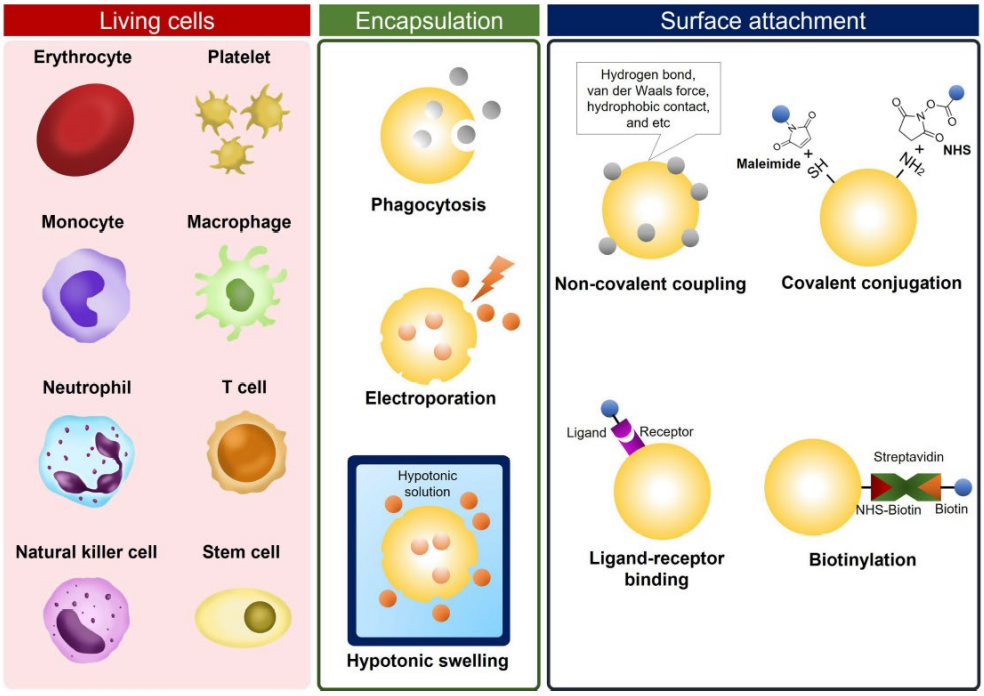

Figure 10- Schematic of living cell-based drug delivery systems. Adapted from (30).

3. Regulatory Approach

In this section is presented a holistic view of the regulatory process required for a medicine to obtain marketing approval in the EU and the United States, as well as the information required for the scientific evaluation of a novel drug intended for human use. An understanding of the current regulatory framework is pivotal so that appropriate considerations and ideas can be provided regarding the development of a framework adapted towards nanomedicine marketing applications, capable of dealing with the unique properties and behaviour characteristic of nanomaterials.

3.1. EU Marketing Authorization

3.1.1. Marketing Authorization Procedures

Marketing authorization for human use pharmaceuticals is regulated by EMA and its scientific committees. There are 7 committees, 6 of them involved in the evaluation of human medicines, as visible in Figure 11.

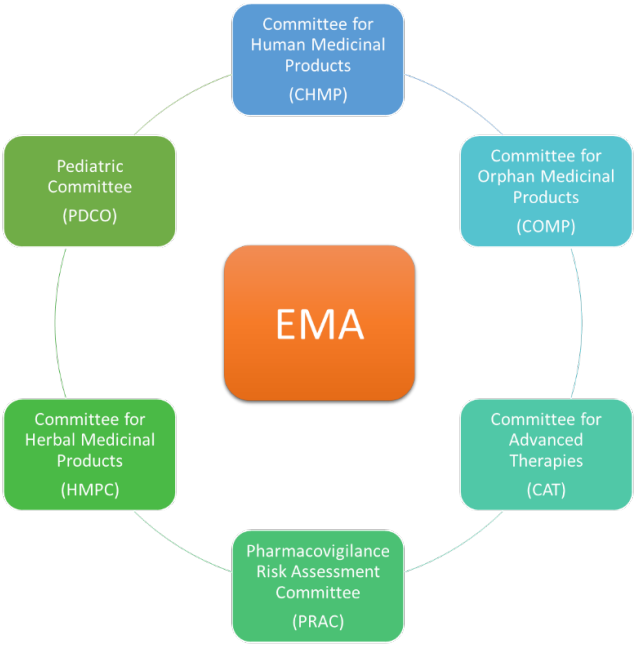

Figure 11- EMA Scientific Committees involved in marketing authorization evaluations for human use medicines.

Marketing authorization procedures can be grouped into two categories. Firstly, the centralized procedure (CP), where authorization is granted in all the EU member states (including Norway, Iceland and Lichenstein), and secondly the non-centralized procedures, resulting in a national marketing authorization in one or more member states involved in the procedure. Each category has different legal provisions and may be handled by national authorities. Figure 12 displays the different types of MA procedures (31).

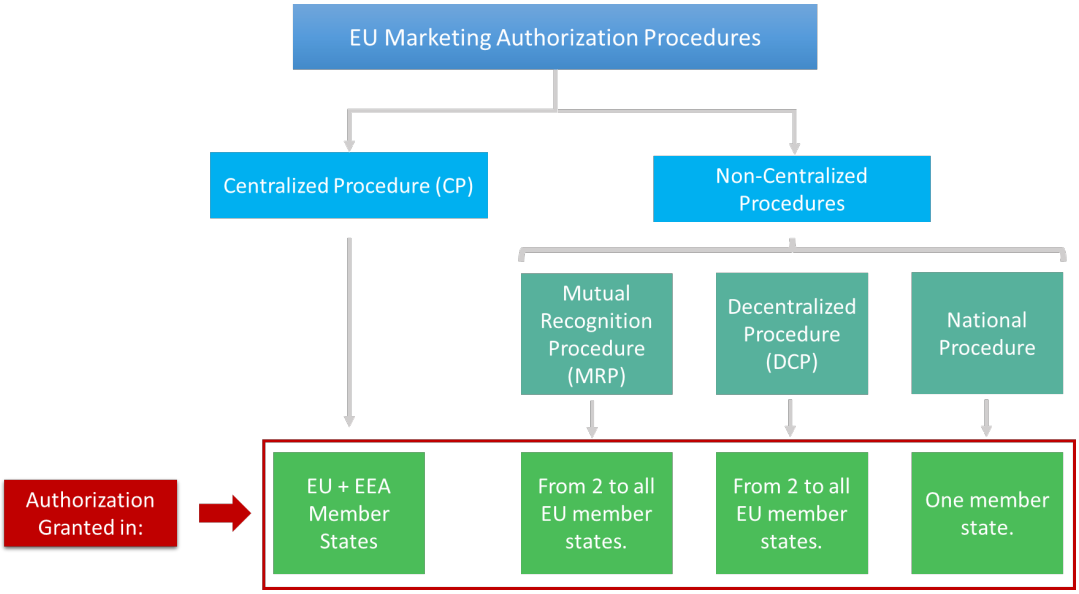

Figure 12- Different types of MA procedures available in the European Union.

The development of a new medicinal product involves several phases, however, from a regulatory standpoint the most important is the Marketing Authorization Application (MAA) Evaluation. Regarding centralized procedures the evaluation is largely carried out by the Committee for Medicinal Products for Human Use (CHMP) with support from the Pharmacovigilance Risk Assessment Committee (PRAC), Committee for Advanced Therapies (CAT), Committee for Orphan Medicinal Products (COMP) and external scientific advisors when deemed necessary (32). For non-centralized procedures the evaluation may be assigned to national agencies following the guidelines from EMA and the relevant committees.

EMA is solely responsible for the CP, which is mandatory for novel active substances intended for the treatment of human immunodeficiency virus (HIV) or acquired immune deficiency syndrome (AIDS), cancer, diabetes, neurodegenerative diseases, immune dysfunctions, viral diseases, biotechnology-based products, advanced therapy medicines (ATMPs) and orphan medicines. This route is also an option for human medicines containing novel active substances for different indications than those referred as mandatory, and considered of significant therapeutic, technical, or scientific significance towards the interest of patients at the Union level (33).

Since most nanomedicines are directed towards the mandatory diseases group, the focus of this work was directed towards the centralized procedures, for which EMA and CHMP are responsible. The structure and timeline of the Evaluation step for CPs is visible in Figure 13.

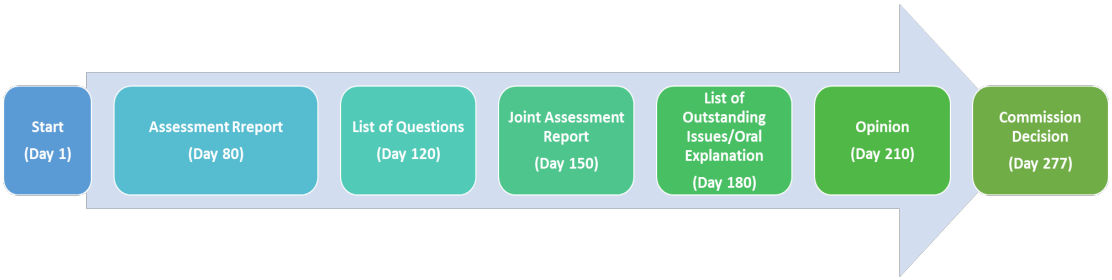

Figure 13- Timeline for the evaluation of a MAA.

After the technical validation of the MAA by EMA, the CHMP initiates the scientific evaluation of the submission. The entire procedure may take up to 210 active days. From day 1 the CHMP rapporteur and co-rapporteur start an independent assessment of all the evidence supplied by the applicant in the application submission to provide the first assessment reports. Both reports contain the scientific evaluation of the data provided, along with any shortcomings, limitations and errors identified. In addition, a list of questions for the applicants to respond is also issued. Besides the scientific evaluation, the risk management plan of the applicant is also evaluated by the PRAC appointed members. All the drafted reports are analyzed by peer reviewers appointed by the CHMP, providing additional comments and questions. At this stage additional entities can be consulted if appropriate. By day 120 a final assessment report is provided to the applicant containing a general assessment of the submission, as well as a list of questions to be addressed (34).

At this stage the evaluation is paused, designated the first clock-stop, and 3 to 6 months are usually provided to the applicant to respond to the question list and provide necessary updates to the submission. When the information is provided, the evaluation is resumed, and the rapporteur and co-rapporteur consider the received responses to provide an updated assessment report. By day 180 the final assessment report along with existing outstanding questions and issues are provided to the applicant. If necessary, a new clock-stop is taken at this stage, and the applicant benefits from 1 to 2 months to reply. A session for oral explanations may be conducted. At this stage any CHMP member can consult with external working parties, including patients and healthcare professionals.

The provided revised information is evaluated by the CHMP rapporteur and co-rapporteur and the PRAC members, reviewed in a joint meeting and, at the latest by day 210, the CHMP adopts an opinion on the MA and makes a recommendation on whether the authorization should be granted and under which conditions. The applicant has a period to appeal the recommendation. By day 232 EMA must be in the possession of the Summary of Product Characteristic (SmPC), labelling and PL

documents in the EU official languages and by day 237 all the final data is transmitted to the European Commission. After consideration, by day 277 the EC either approves or refuses the marketing authorization (34).

Depending on the type of medicine, the intended therapy use, the manufacturing procedure among other factors, some exceptions and deviations from the standard centralized procedures may be adopted. One example is for orphan medicines developed for rare diseases for which incentives for approval exist (35).

3.1.2. Required Information for Marketing Authorization

In terms of the data to be provided by the applicant in the MAA, the document to be submitted is very clear and has a predetermined structure to be followed by the applicants. After the initial submission is validated, the applicant is required to submit the Common Technical Document (CTD), which is divided into five modules. Modules 3 through 5 are organized according to Guidelines M4Q (36), M4S (37) and M4E (38). The CTD is common to all the MAAs independent of type, and the body of the CTD is common to the EU and the USA, following International Council for Harmonization (ICH) guidelines.

- Module I: Contains region specific documents regarding administrative information and prescribing information.
- Module II: Module II is labelled as “Common Technical Document Summaries” and must address 7 sections: CTD Table of Contents, CTD Introduction, Quality Overall Summary, Nonclinical Overview, Clinical Overview, Nonclinical Written and Tabulated Summaries, and Clinical Summary.
- Module III: Quality.
- Module IV: Nonclinical Study Reports.
- Module V: Clinical Study Reports.

The guidelines pertaining to modules III through V of the CTD are very specifically structured. For the Quality module III, the document is structured by ICH M4Q, guided by ICH Guideline Q6A, which has the purpose of assisting, to the maximum possible extent, the definition of a universal set of specifications for approval of new drug substances and products. This specification set includes tests, analytical procedures, and acceptance criteria to which a new drug must conform for it to be considered of sufficient quality to be accepted for the proposed use. Quality and consistency of a product are ensured via the application of specifications to design, development, in-process controls, process validations, while also complying with Good Manufacturing

Practices (GMPs). It is stated in the document that its contents are not encompassing and rigid, that new procedures and technologies should be used if justified.

ICH topic M4S pertains to the non-clinical study reports, Module IV, and the Nonclinical Overview and Nonclinical Written and Tabulated Summaries to be included in Module II. The Nonclinical Overview includes the evaluation results of the pharmacologic, pharmacodynamic and toxicologic evaluation of the pharmaceutical. The nonclinical testing strategy and analytical methods applied should be discussed and justified, while following relevant guidelines on how to conduct such studies whenever those are available. A connection with the results obtained in clinical evaluation and quality assessments should be disclosed. Impurities, limits to impurities and degradants should also be tested and justified, as well as a study on the influence of potential changes to those parameters between the tested and the marketed final product. In this part it is of utmost importance to disclose onset, severity and duration of adverse and toxic effects. The dose dependency, reversibility and other relevant factor should be evaluated. A complete list of such factors is available in the document. A baseline structure for the Nonclinical Written and Tabulated Summaries is provided in ICH M4S. For pharmacodynamics and pharmacology ICH S7A (39) serves as guidance for the design of *in-vivo* and *in-vitro* test systems. Pharmacokinetics summaries involve a description and justification of the analytic methods used, as well as the results for Absorption, Distribution, Metabolism and Excretion (ADME), as well as potential interactions and other studies considered relevant by the applicants. Toxicological assessments should address Single-Dose Toxicity, Repeat-Dose Toxicity, Genotoxicity, Carcinogenicity, Reproductive and Developmental Toxicity, Studies in Juvenile Animals, Local Tolerance and Other Toxicity Studies. Module IV must contain detailed results of the aspects discussed above.

ICH M4E details how the Clinical Overview and Clinical Summaries should be presented in Module II, as well as the entire Module V dedicated to Clinical Studies. Clinical study reports are devised using guidance from ICH E3 Guideline (40). The guideline guides applicants in the creation of a clinical study report which is complete, free from ambiguity and organised, containing relevant results, demographic selection criteria and appropriate statistical support. Module V contains information regarding biopharmaceutic studies, studies Pertinent to Pharmacokinetics using Human Biomaterials, reports of human pharmacokinetic studies, reports of human pharmacodynamic studies, reports of efficacy and safety and finally, reports of post-marketing experience. The clinical overview is a reference to the overall clinical results and should summarize the findings to aid in the evaluation process. It should highlight the strengths of the product, mention the limitations and perform a risk benefit analysis.

The clinical overview should describe the overall clinical approach followed, evaluate the design and performance of the conducted studies, provide a general view of the findings and limitations, assess the benefits and risks of the product based on the outcome of the clinical trials, considering the efficacy and safety discoveries as well as a justification for the prescription information. The Clinical Summary is meant to supply a summarisation of all the clinical information in the Common Technical Document.

3.2. USA Marketing Authorization

The submission process of a New Drug Application (NDA) for the FDA is very similar to that of a MAA to EMA, in the way that the submission must be prepared using the same CTD, submitted via the electronic format. This implies that the same information discussed in the previous section for Modules II to V must be submitted to the FDA, while Module I is different and specific to the USA.

The review process of the NDA is the fourth step in the overall approval process for a novel human-use drug, following the pre-submission activities, process submission and review planning. Following the review process, steps 5 and 6 correspond to making an official decision and post action feedback, respectively (41). An approximate timeline of the tasks described is available in Figure 14.

Figure 14- Approximate timeline for the evaluation of a NDA marketing introduction in the USA, regulated by the FDA. Adapted from (41).

The review process is structured differently than that described for the EU. The FDA appoints a review team responsible for the evaluation of the submission, constituted by different members with distinct functions and a clear hierarchy. The structure of the team is represented in Table 2.

Table 1- Structure, members and respective functions of the review team appointed by the FDA. Adapted from (41).

Summary of Review Team Responsibilities

Primary Reviewer	Discipline Team Leader (DTL)	Cross-Discipline Team Leader (CDTL)	Regulatory Project Manager (RPM)	Discipline Director	OND Division Director (Includes Deputy)	OND ODE Director (Includes Deputy)
• Performs scientific review, labeling review, and recommends action. • Consults with TL, peers, and others while developing the review. • Works collaboratively within a team setting. • Raises issues and identifies to management potential solutions throughout the review. • Attends and participates in review team meetings as needed. • Organizes work to meet deadlines.	• Assigns and provides guidance and feedback to primary reviewer. • Provides clear direction to the primary reviewer; meeting regularly to provide feedback and discuss issues. • Resolves conflicts related to discipline area. • Attends review team meetings, as needed. • Attends and participates in key milestone team meetings. • Advises CDTL of potential schedule slippage and issues. • Signs off on primary review and writes a DTL secondary review, as needed. • Organizes work to meet deadlines.	• Provides day-to-day leadership to team and oversight of the review. • Works with the RPM and DTLs to address issues and resolve conflicts that arise within and across disciplines and to ensure efficient and timely reviews. • Attends all team meetings. • With RPM, monitors review progress and keeps OND management apprised of review status. • Writes a CDTL Review for each application bringing together highlights and perspectives of all disciplines. • Organizes work to meet deadlines.	• Serves as regulatory leader for review team. • Performs PLR format review of label and includes deficiencies in 74-day letter. • With the CDTL, manages day-to-day review activities. • Organizes and attends all review-related meetings (usually facilitates meeting). • Tracks review progress, addresses potential review process issues, and resolves obstacles, keeping the CDTL informed. • Serves as the point of contact for communication with the applicant. • Maintains an accurate administrative record of the review. • Organizes work to meet deadlines.	• Is responsible for ensuring the quality and consistency of the discipline's review and recommendation for action. • Attends key milestone team meetings, as needed.	• Has signatory authority for non-NME applications. • Attends key milestone review team meetings. • Is responsible for ensuring the quality of the review decision, approved labeling, and associated administrative record. • Appoints and mentors the CDTL and works with the CDTL and RPM to ensure that review goals are being met. • With the CDTL handles conflicts that arise during the review. • Writes a tertiary "summary" review that includes a decision or recommendation for regulatory action. • Keeps Office Director apprised of review status and significant issues and organizes work to meet deadlines.	• Has signatory authority for NMEs, original BLAs, and other applications such as novel public health issues. • Attends key milestone review team meetings. • Writes a decisional memorandum for applications. • With the Division Director, ensures the quality of the review decision, approved labeling, and associated administrative record. • Organizes work to meet deadlines.

After submission of the application the review team starts a meticulous analysis of all the information provided. As it can be seen in Table 2 constant communication is maintained throughout the review process between primary reviewers and team leaders. Contrarily to the European procedure, where a list of questions is submitted to the applicant at a predetermined time, the FDA review team maintains contact with the applicant and requests information on a regular basis. Usually around month 5 for regular submissions, or month 3 for prioritized submissions, the Mid-Cycle Meeting takes place. This meeting is held to discuss the current status and findings of the review process, confirm if an advisory meeting is necessary, identify and discuss notorious impediments to the approval, revise the review plan, discuss the risk management plans and discuss labelling (41).

Similarly to the process adopted by EMA, there is the possibility of having expedited and accelerated review processes if the required criteria are met. Nevertheless, these are out of the scope of this report. An important factor to mention is that the FDA has a programme in place designated as PDUFA (Prescription Drug User Fee Act) which consists of an agreement between the industry parties and the FDA, where the manufacturers agree to a fee system directed towards funding the regulatory activities of the FDA, receiving in return an agreement to a set of performance goals by the FDA. One example would be the assurance of a high percentage of applications being reviewed in under a specified timeframe. For the applicants enrolled in the programme some benefits are rewarded in the review process. More information can be consulted in (42). A similar programme is available for review and approval of biosimilars, BsUFA (Biosimilar User Fee Amendments) (43).

4. Nanomedicine and Regulation: Past to Present, USA and EU

Due to the complex properties and behaviour of nanomedicines owed to their dimensions, it was quickly understood the necessity of a specific regulatory framework. The first nanomedicine approved by the FDA was Ambisome, a liposomal amphotericin B, in 1990, which was followed by Doxil/Caelyx in 1995, this being a liposomal drug conjugate indicated for cancer treatment (44). At that time, the regulatory agencies had no provisions for regulatory guidelines aimed at nanomedicines, a scenario that was maintained until 2006, when both in the United States (US) and EU published documentation addressing this issue. Until some guidance was published, the regulation and approval of nanomedicines followed the trends of already established guidelines for existing medicinal products.

4.1. United States Regulatory Timeline

In 2006 the US Food and Drug Administration (FDA) established the Nanotechnology Task Force in recognition of the advances in nanotechnology and their relation towards pharmaceutical use. The Task Force published their first report in 2007 (45) and addressed scientific and policy issues regarding products containing nanoscale products. The authors main findings in terms of scientific issues relate to the possibility of biological interactions and the adequacy of the testing methods to assess safety, quality and effectiveness of products containing materials at the nanoscale. The need to establish standardized characterization methods, testing methods that account for the specific properties of nanoscale materials, collaboration protocols between regulatory agencies, manufacturers and academics to increase information flow and transparency, among others were key points highlighted by the

authors. In terms of policy, the report clearly identifies the need of regulation regarding nanoscale materials.

Later in 2007, the National Nanotechnology Initiative (NNI) published a document regarding the US strategy for research targeting nanotechnology and the environmental, health and safety concerns (46). This document evaluated several aspects regarding the future strategies for nanotechnology research and applications with basis on published documents, and identified several aspects that should be implemented towards bettering the research approaches on nanotechnologies. Again, the importance of precise, innovative and standardized testing procedures for nanomaterials and their interaction with humans and their biological systems was pointed out, as well as the need for research to take into consideration risk assessment and the importance of collaboration and flow of information. Nevertheless, despite the document providing some principles on how nanotechnologies should be developed and implemented, the document was criticized by lack of specificity, insufficient material regarding risk assessment and minimization strategies, low emphasis on exposure assays and the need for higher funding (47).

These documents successfully recognized the immense potential and growth of nanotechnology and its implementation across all sectors of society, with the respective concerns associated with that implementation. However, criticism from stakeholders pointed towards the fact that the FDA stated at the time that existing regulations and guidelines for pharmaceutical products were sufficient and adequate to evaluate nanomedicines. This affirmation was considered as dangerous by experts, seeing that the common approach was approval by comparison with non-nano versions of similar products, which can result erroneous due to the unique properties of nanomaterials.

In 2014, the FDA recognized the existent void in the classification of nanomaterials and their lacking definition of such materials, consequently leaving doubt over which products regulatory activity should be enforced. In that sense, the "Considering Whether a FDA-Regulated Product Involves the Application of Nanotechnology" was issued and, the criteria followed by the FDA to consider a product as containing nanotechnology was based on two aspects (7). Firstly, if the material or the end product was engineered to have one of its characteristic dimensions in the nanoscale range, comprehended between 1 and 100nm, and secondly, if the material or end product exhibited properties or phenomena attributable exclusively to their dimensions, in a scale up to 1000nm. Again, the document contained some remarks regarding safety and testing, but the regulatory framework for nanomedicines

was still inexistant and analysed in a case-by-case manner following available medicine guidelines.

Also in 2014, the FDA published a document addressing the specific application of nanoparticles to cosmetic products and the safety associated with these applications, motivated by concerns expressed in the 2007 report from the Task Force (48). The motivation behind these specific guidance reports is the fact that at the nanoscale, properties such as the chemical, physical, optical, electrical and biological may suffer significant and relevant alterations from the larger counterparts. This report includes remarks on how the toxicity testing methods should be modified to account for property changes and for the complete characterization of the nanomaterials involved in the process. In 2014 and 2015, two guidance documents were issued regarding the application and testing of nanoparticles in food ingredients (49) and in food for dogs (50).

In 2017 the initial draft guidance for industry was published by the FDA (51), and the document was subject to analysis by several institutions and stakeholders which, despite welcoming the documentation, addressed several key points that helped the FDA in developing the final version of the document, released in 2022. The objective of this document is to understand the current state of the regulation scenario for nanomedicines, therefore, the most recent document will be reviewed below.

In April of 2018, the first class-specific regulatory document for Liposome Drug Products was released in the US by the FDA (52). The document addresses several important factors and properties that are specific to liposome nanomedicines. Aspects such as manufacturing, pharmacokinetics, bioavailability and labelling are included in the report, however, no remarks are present regarding efficacy, toxicity and safety. In a liposome drug product, the drug substance is contained in the liposome, either encapsulated or intercalated (the former refers to substances within an aqueous space and the latter to incorporation of the drug substance within a bilayer). When submitting a NDA or an abbreviated new drug application (ANDA) the applicants are advised to include detailed information on the composition and individual description of the drug product components, either being drug, lipid or nonlipid. The way this information should be presented is also described. A list of relevant physicochemical properties of both the liposomes and drug products is presented in the document and the applicant should address these in the application with support from development tests and data. The surface characteristics of the liposome and particle size distribution are two of the mentioned properties, and these are of particular importance for nanomedicines as discussed in the introductory section. It is recommended that a detailed manufacturing process flow diagram and description of each step is included. The process and

mechanism of liposomal drug loading and removal of free drug from the liposome formulation by purification should be described in detail. The manufacturing process should be validated to demonstrate consistency and reproducibility prior to commercial distribution. Operating parameters should be justified. Particular attention is also given to the manufacturing, specifications and stability of the lipid components.

The pharmacokinetic properties should be evaluated following recommended *in-vivo* studies and guidelines on generic liposome drug products should be consulted. When a non-liposome formulation has been previously approved, comparative studies must be performed and submitted.

In 2022, the FDA issued the final guidance for industry for drug products containing nanomaterials (53). This document provides manufacturers and applicants with key considerations for drug products, including biological products, intended for human use. As per the 2014 version, the FDA will determine whether a product regulated by the FDA involves nanotechnology by considering whether the material or end product has at least one external dimension or internal or surface structure in the nanoscale range (approximately 1 nm to 100 nm). Additionally, the FDA will also evaluate whether a material or end product exhibits properties or phenomena that are attributable to its dimensions, including dimensions outside the nanoscale range up to one micrometre (1,000 nm), which are relevant to safety, effectiveness, performance, quality, public health impact, or regulatory status of products. Despite being specific towards drug products containing nanomaterials, the document still does not provide a standardized methodology for testing and characterizing nanomedicines. Issues such as safety, effectiveness and public health impact are still to this day treated on a case-by-case approach using existing methodologies.

Given the wide variety of nanomedicines, in the way they are manufactured, administrated and structured, the FDA considers that a regulatory framework for this type of materials must assure two critical aspects. Firstly, adequate characterization of the nanomaterial and secondly, an adequate understanding of the intended use and application of the nanomaterial, as well as how intrinsic attributes of the material relate to the product. A risk-based approach should be taken, focusing on specific risk factors that are addressed in the guidance document. Manufacturers of drug products containing nanomaterials should utilize information gathered throughout the product's life cycle to continually reduce residual uncertainty, as with all drug products.

The quality recommendations present in the document address the description of the nanomaterials in the drug product, which should evolve along the product development, the nanomaterial critical quality attributes (CQA) and structural characterization, which should be determined with basis on type of nanomaterial,

function, route of administration, and impacts on performance along with a sensitivity analysis on property changes. Aspects regarding standardized characterization methods and their adequacy and justification are presented, as well as criteria for presenting a fully validated dissolution/*in-vitro* release method, whether being common or newly developed, to ensure quality and clinical performance. For nanomaterials, because of the increased dependency of the properties on the manufacturing parameters there is not available at the moment a thorough database on the influence quality aspects play on the quality and manufacturing of drug products. Hence, it is important that manufacturers build experience over time and with the maturing manufacturing process to develop an understanding of potential risks associated with critical quality aspects. The document highlights the importance of establishing CQAs early in the development phase to develop adequate in-process controls to detect manufacturing failures and defective samples.

When nanomaterials are employed as excipients proper controls, including testing and failure criteria, full description of the material and source must be specified. If there is documented prior use in humans under similar and relevant conditions a brief description may be accepted. If an excipient is deliberately transformed into a nanomaterial, the original properties cannot be assumed as preserved and the FDA regulation on the safety of new excipients must be followed (54). The current guidelines established for stability criteria, ICH Q1A (55) for drug products applies to those including nanomaterials. For these it is important to assess any property changes during handling and storage, namely interaction with packaging surfaces or contact with administration or delivery devices. Additional testing is required.

Given the strong influence manufacturing parameters play on the critical quality attributes of nanomaterials, when post-marketing changes are being submitted, a thorough comparison between physicochemical properties and bioequivalence studies may be required. Preservation of samples from fundamental batches through development to enable bridging between manufacturing conditions and final attributes is a good strategy for manufacturers.

Concerning non-clinical studies, the recommendations and guidelines established by the ICH are generally applicable to nanomedicines. Special considerations should be taken when evaluating ADME, as well as pertinent risks associated with specific administration routes. Examples are provided in the document to guide manufacturers in evaluating their products and the necessity of special considerations.

For the clinical development of drug products containing nanomaterials, the document addresses specifically the development of drug products in reference to an

already approved drug or a particular reference drug. For the FDA specifically, there are 3 submission categories that apply to this scenario, being 505(b)(2) for NDA, 505(j) for ANDA and 351(k) for Biologics License Applications. Regarding 505(b)(2) submissions, some steps are simplified when compared to a fully new drug submission. From a pharmacokinetic-pharmacodynamic (PK-PD) perspective there are two types of nanomedicines. Firstly, those where the nanomaterial is the therapeutic ingredient and secondly, those where the nanomaterial acts as a carrier for the API. Examples of the second category are liposomes, polymeric nanoparticles and dendrimers. Nanomaterial carriers can enhance the delivery of the API by various routes, such as the endocytic route or the enhanced permeation retention (EPR) effect, therefore, the disposition and exposure-response relationship for the drug product containing nanomaterials may differ from the reference drug not containing nanomaterials. Bioavailability equivalence studies and additional evidence may be required to demonstrate suitable disposition and exposure-response relationships. For development programs attempting to bridge the performance of the proposed drug and a reference drug, the FDA recommends a risk-based assessment, with the evaluation of the risk the product being developed having different properties regarding exposure, safety and effectiveness. The risk is dependent on certain characteristics of the drug product, route of administration or frequency of use. The FDA lists examples of drug product properties that are illustrative of risk categories to aid in the decision of the manufacturer when evaluating to what extent their development program must prove the equivalence between the development drug and the reference one. Depending on the risk category, the necessary clinical and equivalence studies are highlighted. For example, for a low risk drug, a plasma pharmacokinetic study to demonstrate comparable bioavailability may be sufficient. For medium and high risk products, single and multiple-dose ADME studies to evaluate PK-PD are recommended with appropriate biomarker selection.

Regarding the 505(j) submissions for a generic product referencing a drug product containing nanomaterials, an ANDA can be submitted if there is bioequivalence (BE) evidence. Because of the wide variety in nanomaterial formulations, administration routes, release mechanisms and biodistribution, similar PK parameters in plasma studies might not be enough evidence to support BE between the generic drug product and the reference medicine. The FDA proposes in the document BE studies that should be employed according to the administration route intended for the generic product.

Finally, for 351(k) submissions involving biological products the FDA states that general biosimilar guidance documents should be followed (56),(57),(58).

Regarding *in-vitro* Tests for clinical development using Human Biomaterials the FDA lists stability and biocompatibility, plasma protein binding, clearance, and metabolism as critical assays to perform. Finally, some considerations regarding environmental impact are presented.

By publishing this document, the FDA addressed key aspects that manufacturers and regulatory agencies must prepare for when developing drug products containing nanomaterials. Despite not being a specific regulatory framework that establishes a clear strategy and pathway to be followed, it is a significant aid in simplifying the approval process.

This document is the result of several years and efforts toward preparing staff, infrastructure and research capacity. Since the establishment of the Nanotechnology Task Force (NTF) in 2006 significant progress has been achieved in nanotechnology-related research including regulation. In 2013 the Nanotechnology Regulatory Science Research Plan (59) was presented, intending to fill existing gaps in nanotechnology regulation science through incentives in infrastructure, collaborations with other agencies and specific staff training. FDA Collaborative Opportunities for Research Excellence in Science (CORES) were set up, with two facilities exclusively dedicated to nanotechnology. Through collaboration with other US government agencies, the National Nanotechnology Initiative (NNI) (60) was established in 2011 which addressed 6 main areas: physicochemical characterization of nanomaterials, nonclinical models, risk characterization, risk assessment and risk communication. These areas were identified by the CORE programs and led to the development of guidance for industry documents.

4.2. EU Regulatory Approach

Noticing the advances in nanotechnology and the strong potential for a wide variety of applications, the European Union held a communication on the 12th of May 2004 (61) addressing the hypothesis of developing a strategy for Nanotechnology and Nanomedicines (N&N) to strengthen the position of the EU in becoming a strong figure in the field. In the conclusions of this communication the idea was accepted and, in 2005, the “Nanosciences and nanotechnologies: An action plan for Europe 2005-2009” was published with the aim to provide immediate measures for a safe, integrated and responsible strategy for N&N (62). The first talking point of the plan is addressing the necessity of information and research and development (R&D) on the topic. For that, incentives for collaborative programs of research, not only at program levels, but also at European levels, have been set up, with an increase in the budget allocated to the topic. The necessity of research and studies on the impact of these nanomaterials on

human health was also one of the first priorities established by the EU. Secondly, some measures were implemented to ensure that the EU formed a consistent system of an infrastructure composed by single sites and networked facilities, integrating both public and private R&D centres to maximize the available resources. To exploit the excellent facilities, the EU recognized the need for training and forming highly skilled human resources by implementing workshops and educational programmes, incentivizing research in the area with awards and stimulating universities and industries to push the boundaries of the topic and improve the quality of education. The importance of delivering the produced knowledge to practical marketed applications was highlighted, as well as the need to establish a harmonised and optimized system to ensure intellectual property rights (IPR). At the time, the Commission fully recognized the potential for issues and risks associated with nanomaterials. Measures to ensure and encourage an open dialogue regarding issues related to N&N were suggested, as well as maintaining responsible, ethical and sustainable R&D strategies. Moreover, the necessity of regulation and risk assessment strategies to control public health and environmental impacts and hazards was also recognized. The results of the action plan were analysed and reported in a final communication issued in 2009, where the main achievements are highlighted (63). Some of the important accomplishments are the establishment of the ObservatoryNANO (64), a nanotechnology observatory, the adoption of a code of conduct associated with nanoscience and nanotechnology R&D, and the adoption of a communication regarding regulatory aspects of nanomaterials (65). In 2014 the EU officially defined a nanoparticle in a communication as a “natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1nm to 100nm” (66). In October 2012 a second communication regarding the regulation of nanomaterials was issued (67), referring to the adequacy of the EU legislation at the time and issues raised by European agencies. In this communication is stated that, although some specific hazards related to nanoparticles had been registered, the existing regulation on the different sectors was enough to support a case-by-case analysis of nanomaterials. This approach was also followed by EMA for nanomedicines, with 20 medicines containing nanomaterials already approved in 2012.

One of the most important regulatory entities in the EU is the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) (68) which is enforced by the European Chemicals Agency (ECHA). This agency was established in 2007 and is one of the most comprehensive chemical regulations developed. Their

main tasks include coordinating the registration of chemical substances by manufacturers and importers, evaluating the safety of chemicals, and managing the authorization and restriction processes for hazardous substances. REACH is also applicable to substances that have some form in the nanomaterials category. An analysis of the substances containing nanoforms which had been previously accepted by REACH revealed that little to no information was present regarding the safe use of the nanomaterials specifically. This was attributed to a general lack of detailed guidance for registrants on this topic, identifying a clear problem to be attended to in the near future. More information regarding actions, measures and entities put in place around 2012 is visible in the annex of the communication. The communication was also accompanied by a staff review paper (69) which was an extensive compilation of nanomaterials known and approved at the time, their uses and safety data.

In 2014 the results of a research study undertaking a 10-month period comprised between January and December of 2013 were published (70), with the study assessing the impact on the REACH regulation pertaining to Nanomaterials and regulatory options to be adopted in the future to better assess nanomaterials under the REACH regulation. This study involved stakeholders, namely the manufacturers, which are mostly affected by the lack of clear regulation, with dossiers pertaining to nanomaterials often falling short. Results from a Public Consultation revealed that the overall view of current registration provisions for the registration of nanomaterials, 68% considered it to be “unclear” and a further 18% “very unclear”. The study evaluated the competitiveness of the European nanomaterials sector, innovation and employment, including small and medium enterprises’ specific impacts as well as human health and environment. The study concluded that, despite the insufficient clarity, the majority of stakeholders believed REACH to be the most appropriate tool to regulate nanomaterials and that a viable option to improve existing regulation would be to introduce modifications in some Annex provisions clarifying what manufacturers and companies are expected to do in accordance with the registration obligations of REACH and their specific guidance, which takes into account an internal ECHA discussion on how REACH applies to nanomaterials (71) and the REACH Implementation Project on Nanomaterials (RIPoN) reports.

A very important project developed in the EU between 2013 and 2017, with an overall budget of 49.5 million €, was NANoREG (72) which was the first initiative to deliver answers to regulators and legislators in terms of Health and Environmental Safety (EHS) aspects of nanomaterials and a scientific evaluation in terms of testing procedures and data focusing on the REACH regulation. A workflow of the project is presented in Figure 15.

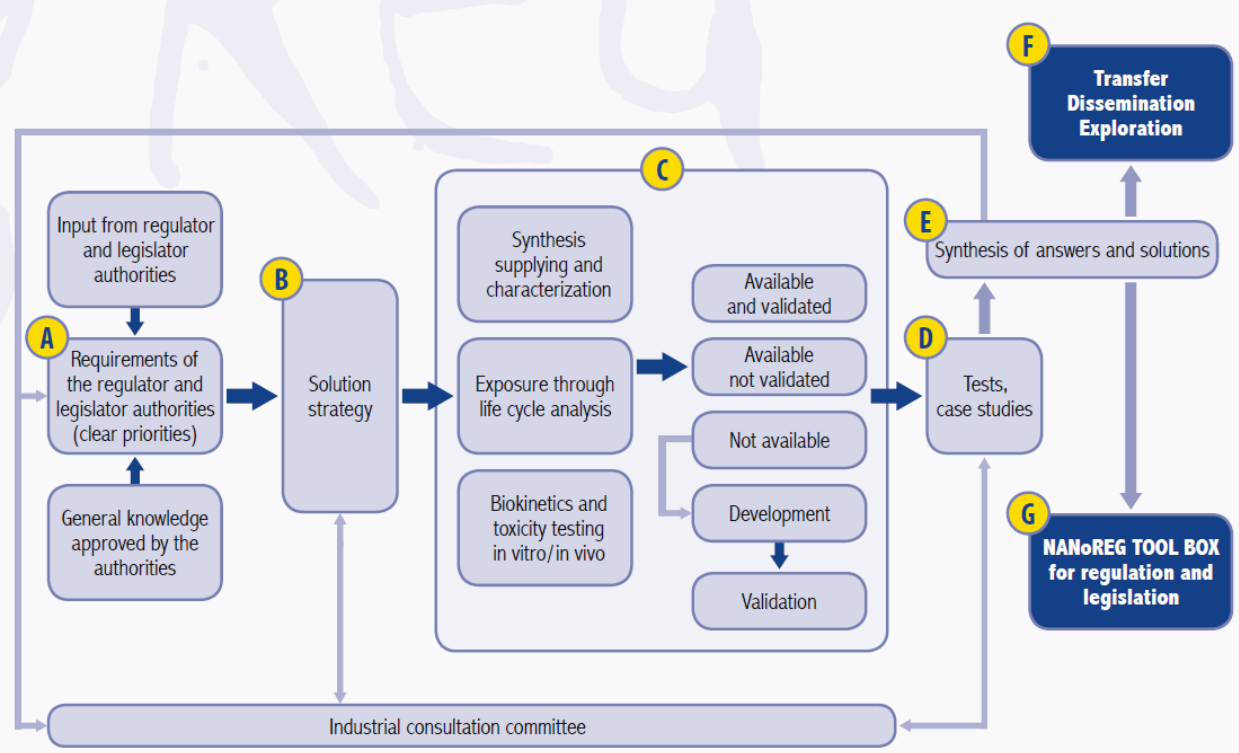

Figure 15- Schematic representation of the NANoREG project workflow. Adapted from (72).

Starting in March 2013 and with a duration of 42 months, involving more than 50 international partners and experts in nanotechnology, this project delivered, among more than 50 publications including guidance documents, scientific papers and standard operating procedures (SOPs) (73), the most important tools for manufacturers and nanomaterial related industries when it comes to regulation. The first is the “Safe-by-Design” (SbD) approach, which ultimately aims at transforming the testing and EHS aspects of nanomaterials from a hurdle to an innovative and evolving process. Such an approach is beneficial due to the significant difference in the technological development and regulation updates time scale. The NANoREG SbD approach was born from the engineering concept of the “stage gate innovation model” which briefly involves distinct stages or phases with defined gates or decision points in between. The safe-by-design approach includes, for the early innovation phases, the identification of potential risks, the calculation of risk indicators in mid-innovation steps and demonstrators of such risks in the later phases, as determined by existing regulation. An overview of the SbD concept is presented in Figure 16, highlighting the main development phases of an innovative product and the steps that should be followed to ensure safe product development and an easier regulatory process upon marketing introduction. Assuming that a risk can be described as the combination of the uncertainty of the occurrence of an event (e.g., exposure to a malicious chemical), expressed as a probability density function, and the uncertainty of the impact said

event may cause, the SbD deals with the identification and evaluation of uncertainties. Risk management encompasses risk assessment (including identification, analysis and evaluation) and risk treatment, for which International Organization for Standardization (ISO) standards exist.

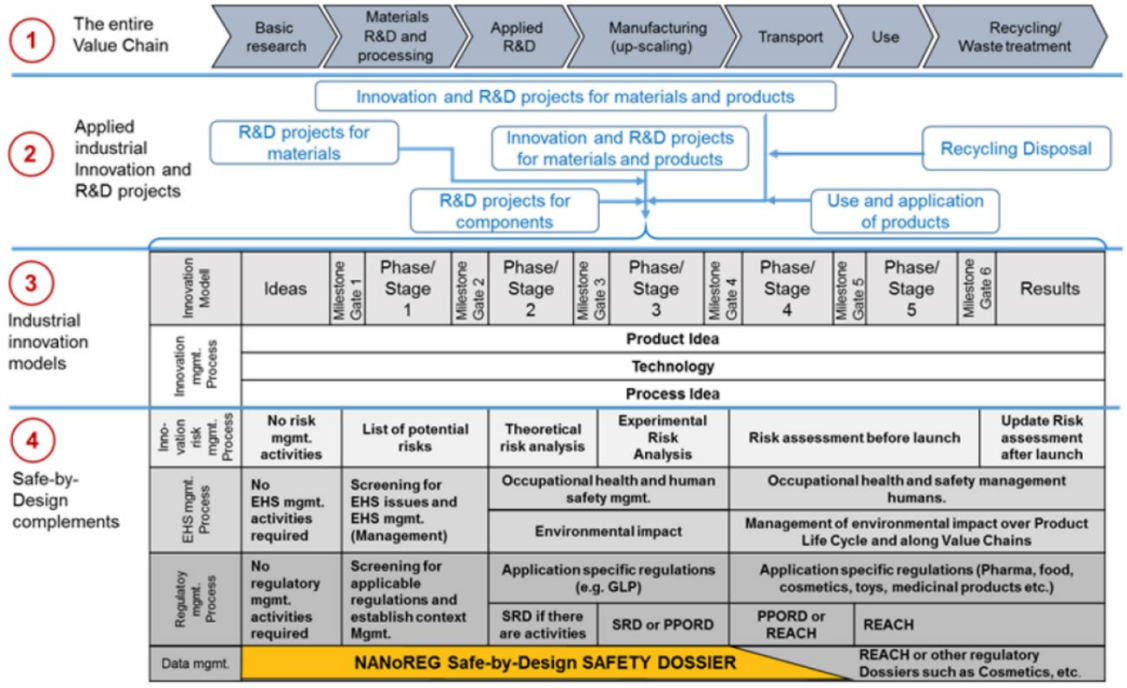

Figure 16- Overview of the SbD approach recommended by the NANoREG for a concise and safe development of new products to facilitate the regulatory analysis. Adapted from (74).

Risks can be categorized according to ISO 31000:2009 and a careful analysis must be performed in terms of the costs associated with risk reduction. On a general basis, the lower the remaining risk the higher the cost, and a total reduction of the risk level is inefficient, therefore, a cost-benefit analysis must be performed. Also, the cost of risk reduction is directly related to the timing of risk identification, the earlier an uncertainty is detected the lower the cost involved and the higher the benefit of investing in risk reduction.

NANoREG also contains a screening strategy intended to identify uncertainties pertaining specifically to nanomedicines, however, this has been revised in NANoREG 2 and will be addressed later. Briefly, six topics are included: exposure potential, dissolution, transformation, accumulation, genotoxicity and immunotoxicity. The second major deliverable of the project was the NANoREG Toolbox (75,76), the most important tool for the successful implementation of the framework proposed in the project. This toolbox contains test methods, datasets, guidance documents, decision flowcharts and other relevant and concrete information that allows the practical implementation of the framework by developers, regulators and other interested

parties. Despite being a major step forward in the regulation of nanomaterials, the NANoREG framework falls short on developing a standard regulatory approach to be followed, instead it is highly developed around the established and existing REACH regulation. Several other European entities are involved in the EHS of nanomaterials (NMs), including the Organization for Economic Co-operation and Developments (OECD) and the ISO which have amounted and published significant guidelines and documents relevant to procedures associated with the safety of NMs (77)(78).

A follow-up on NANoREG came in 2015, NANoREG 2 (79), with the new project ending in 2019 and aiming specifically at the implementation of the SbD framework in the regulatory frameworks. The main objective is to build a regulatory landscape that is flexible and has the ability to deal with and adapt to the rapid innovation and requirements associated with innovative products. This includes the introduction of grouping policies for nanomaterials, which at the time were non-existing and were one of the main needs identified by the regulatory agencies and industries. The main outcome from NANoREG 2 is the Safe Innovation Approach (SIA) (80), which is a combination of the SbD concept with the Regulation Preparedness (RP). The latter was developed so that regulators can be prepared in advance for emerging materials and technologies as soon as the early innovation stages begin. This includes a synergetic effort and communication between developers and regulators throughout the entire process to provides early safety data and assure a flexible and adaptable regulatory approach while ensuring all the necessary aspects. In Figure 17 is schematically represented the SIA framework and strategy.

One of the most important aspects for regulators and addressed by the SIA methodology is safety. In that regard, the relevant safety information necessary for each phase of the innovation process was identified, explicitly for nano-specific environmental and health risks. More concretely, three safety elements are defined: uncertainty, exposure and hazard. Uncertainties are associated with a lack of information, therefore, to reduce the level of uncertainty it is crucial to collect and maximize information associated with nanoforms, whether about characterization, reactivity or toxicity. Standardization methods and data quality are also of great relevance and favours greatly grouping strategies. Exposure reduction measures should be implemented throughout the life cycle of the nanomaterial. Finally, overall risk can be lowered by reducing hazardous properties with the aim for the nanomaterial to be as non-toxic as physically possible. This includes carefully addressing properties such as size, shape, and surface chemistry and employing protectors such as coatings always without compromising functionality below the desired level. With the basis of this information the SbD scenarios were developed according to Figure 18.

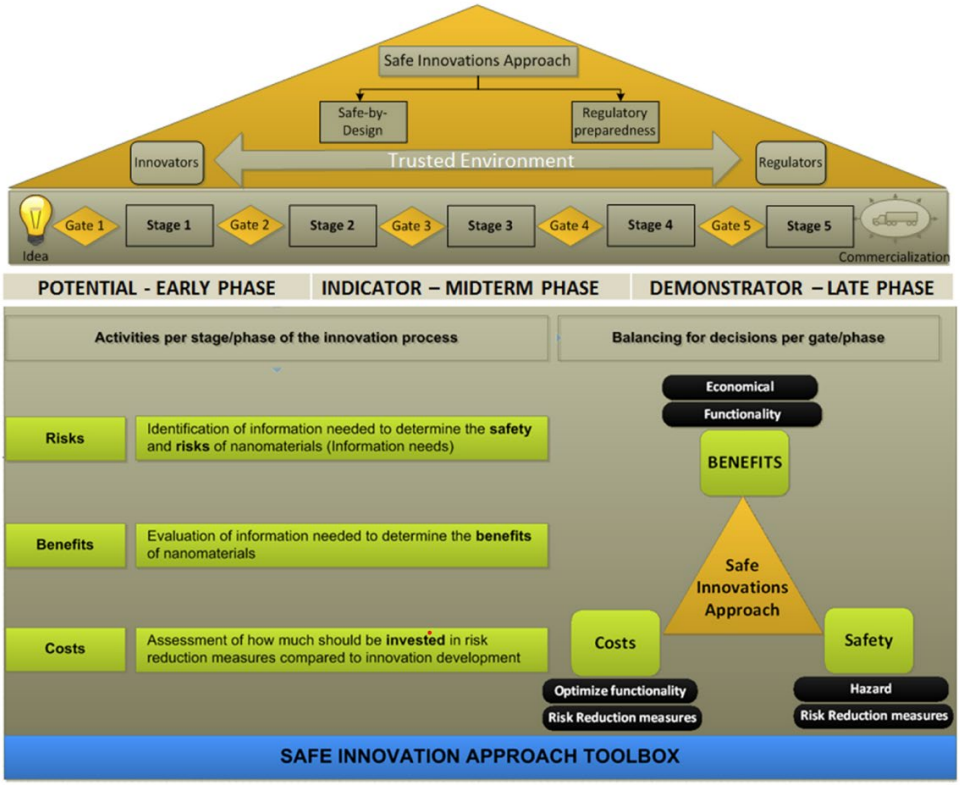

Figure 17- SIA framework schematical representation. Adapted from (81).

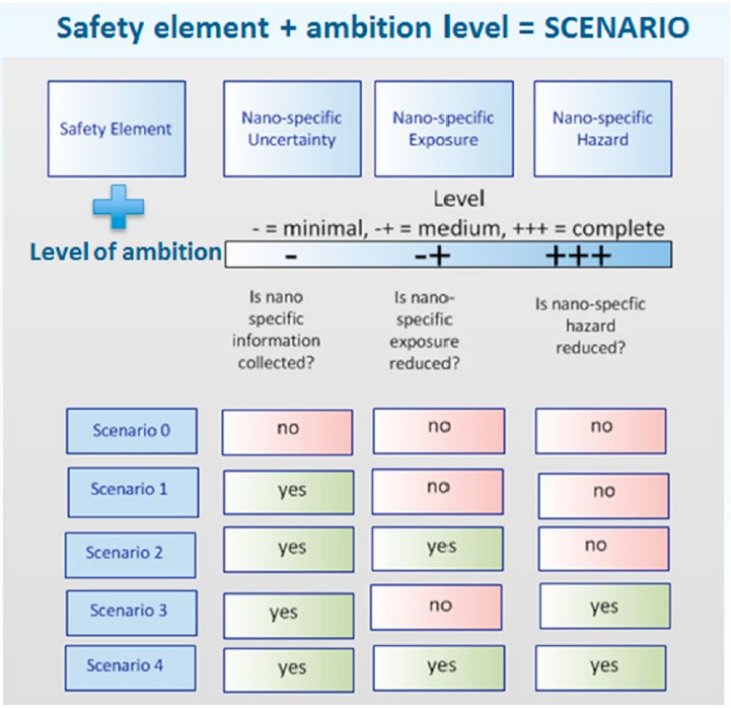

Figure 18- SbD safety scenarios. Adapted from (74).

The SbD scenarios are intended to set safety ambitions and targets which can be recognized by developers and policymakers, as well as serve as a guidance tool for the practical application of the SbD approach. Regarding the different phases of the

development process, NANoREG 2 provides recommendations on the type of information that should be addressed in each phase for risk reduction purposes.
Additionally, for the practical implementation of the SIA both a SIA toolbox and SIA platform were developed. The former consists of a set of tools and guidance documents that aid developers in cost/benefit balance while reducing risks and maintaining functionality. Cost minimization is of particular importance since testing procedures involve high investments, therefore, existing data on similar substances and innovative testing procedures should be available to fill data gaps. This information can be consulted in the NANoREG 2 database and a practical guide on how to combine the toolbox with the database was produced. The list of tools and guidance documents available in the NANoREG 2 toolbox can be consulted in (82).

The fulfilment of existing data gaps in the nanomaterial space is of utmost importance and grouping strategies are useful across the entire innovation phases. Particularly in the early development stages, the selection of a nanomaterial with existing information can provide a grouped regulatory approach in later stages, making the procedure highly efficient. The NANoREG 2 database is the continuation of the previous NanoReg toolbox developed within the FP7 project eNanoMapper (83). It constitutes a library of information regarding nanomaterials including material identity, measured properties (physicochemical characterization and safety related), protocols (e.g., SOPs or standardized test guidance documents). In addition, the database construction allows connection to other information hubs such as external databases for high-throughput and omics studies data, exposure information, and product webpages.

All the tools and concepts presented above rely on the creation of the designated Trusted Environments (TE), which consist of physical and virtual spaces where industry, developers, governments, and regulators can coexist and share knowledge, information, and technologies regarding nanomaterials and innovative products. These should act as a communication hub where constant and proactive dialogue should take place, enabling at the same time the implementation of RP concepts. The fundamental characteristic of these TEs is confidentiality and the assurance of intellectual property rights, hence, there are technical, juridical, and conduct requisites. In this field, EMA has created an Innovation Task Force and a pre-consultation office to anticipate regulatory challenges posed by the industry, promoting technologies and methods, as well as serving as a platform for information exchange between regulators and developers (84). In this sense, regulatory agents transition from a passive agent to a proactive entities with constant inputs and up-to-date with innovation and development.

Significant effort and funding have undoubtedly been directed at the development of regulation and safety protocols towards the use of nanomaterials. From the mentioned projects, in 2018, the EU issued regulatory amendments to Annexes I, III, VI-XI and XII to include provisions concerning the characterization of nanoforms, the assessment of chemical safety and requirements for registration (85).

Regarding nanomedicines and nanobiomaterials medical products and devices, the EU launched the Refine project in December of 2017 with the specific objective of delivering a regulatory framework for stakeholders based on intelligent testing strategies, risk-benefit analysis and a decision support system (DSS) (86). The purpose of the Refine DSS is to assist the preclinical assessment of medical products and devices using the most recent and advanced methodologies available, by implementing intelligent testing strategies (ITSs) that line with the interests of stakeholders and the regulatory agencies demands.

The main deliverable from the project was a Guidance Manual for Stakeholders (87) which outlines the methodology behind the selection of ITSs, the testing methods and how those are to be performed and presented, as well as important recommendations regarding CQA. The DSS clearly distinguishes between Medical Devices (MD) and Medicinal Products (MP) and defines a set of modules according to defining properties for each category. These properties can be contact type and duration for MDs, and administration route or clinical indication for MPs. The modules were constructed following existing ISO and ICH regulations applicable to health products. Another important distinction is made between quantitative and qualitative properties of nanomaterials, the latter are non-measurable properties known a priori which constitute the initial information provided to the DSS. From there, specific assays are applied to determine the relevant quantitative properties, for example, particle size distribution, polydispersity, surface charge among others. The numerical values resulting from the assays are associated with endpoints of the DSS, however, for these values to be converted in results, qualitative classes must be defined so that logical rules and decisions can be established. Such rules are defined by logical operators AND, OR and NOT. A practical example is the applicability rules for the Nanoparticle Tracking Analysis (NTA) assay:

$$\begin{bmatrix} Refractive\ index = low\ RI\ (RI < 1.6) \\ AND \\ Particle\ Size\ Diameter\ (lower\ bound) = 60 \\ AND \\ Particle\ Size\ Diameter\ (upper\ bound) = 1000 \end{bmatrix} OR \begin{bmatrix} Refractive\ index = high\ RI\ (RI \geq 1.6) \\ AND \\ Particle\ Size\ Diameter\ (lower\ bound) = 30 \\ AND \\ Particle\ Size\ Diameter\ (upper\ bound) = 1000 \end{bmatrix}$$

Having defined the knowledge base and the assays to be performed, a prioritization strategy was implemented to guide the evaluation of the Nanotechnology-enabled Health Product (NHP). Therefore, starting with an initial sample of mostly

qualitative information, the prioritized assays are performed and, with basis on the results, a new selection can be executed following an iterative process which consists of an ITS, ultimately gathering sufficient information for the clinical stage. The ITS filters unnecessary assays resulting in an efficient, fast and cost-effective process.

From the literature review it is clear thar significant effort, funding and resources have been placed towards developing strategies for a safe use of nanomaterials in health applications, however, the information pertaining to nanomedicines specifically is still scarce and, at the moment, there is still no specific mention for nanomedicines in the existent pharmaceutical drug regulation guidance documents and guidelines. The approach adopted both by EMA and the FDA is a case-by-case analysis with basis on existent regulation for regular drug products, regular meaning non-nano formulations, focusing on a risk/benefit analysis.

5. Challenges in Clinical Translation of Nanomedicines

Successful development and clinical translation of nanomedicines is dependent and directly related to the available regulation. Ideally, an efficient, fluid, fast-tracking and rapid approval is desired, albeit maintaining the criteria for safety evaluation. This implicates that the regulatory landscape should move and adapt as quickly as the pharmaceutical and technology developments. As simple as the idea is, the problem lies in the implementation due to the nature of nanomaterials. Comprehension of the main factors affecting a successful clinical translation of a nanomedicine is important to understand what underlying factors are hindering advances of these drug products and how regulatory agencies can take a proactive stand to implement information in their guidelines and reflection papers to help manufacturers avoid inefficacious and pointless development pathways, as well as evade common costly mistakes.

5.1. Scale-Up of the Manufacturing Process

As explained in previous sections, at the nanoscale the properties of matter become very sensitive to any procedure or modification. Therefore, it is very difficult to expedite nanomedicine approval without thorough characterization and testing, for which appropriate and standard procedures are inexistent. Not only that, a manufactured nanomedicine or nanoconjugate may present completely different pharmacodynamic (PD) and pharmacokinetic (PK) profiles when compared to those of the constituent materials (88), leading to different biodistribution, exposure, and toxicity among others.

Another major factor to take into consideration in the development of nanomedicines is the transition from small-scale laboratory batches to an industrial

commercial level involving large quantities. The transition must ensure GMPs which are responsible for the quality of processes and end products. The European GMP guidelines (89) are responsible for ensuring quality in the manufacturing of active pharmaceutical products at all levels, including personnel, buildings and manufacturing facilities, equipment, substances and materials, production and in-process control, laboratory controls, packaging and labelling, validation and, importantly, change control. The last variable is particularly important for nanomedicines, given that changes in manufacturing procedures can result in undesirable property changes. It is imperative that the resulting product is reproducible to a very tight standard with variability limited to a very low percentile. These analytical and manufacturing demands often cause innovative projects to fail, given the increase in complexity and demand of the characterization and evaluation procedures experience along the development process. Therefore, it is a very difficult decision for manufacturers to pursue with the development and such decision must depend on the benefit and added value that the product brings to the market and the patients (90).

5.1.1. Quality by Design

The diversity of nanomaterials and nanomedicines adds a complexity degree in the development process and, most importantly, to the definition of quality parameters and attributes to constantly monitor to ensure quality. The FDA in 2004 and the ICH in 2008 implemented a risk-based approach to ensure the quality of manufactured drug products entitled Quality-by-Design (QbD). This philosophy is implemented throughout multiple ICH guidelines for pharmaceutical development, risk management, and quality systems (91),(92),(93),(94). One of the critical aspects of this philosophy is the definition of CQA, which can be defined as a physical, chemical, biological or microbiological property or characteristic that must imperatively lie inside a predetermined limit, interval or distribution for the product to be considered of quality (95). Alongside the definition of CQAs for the substances, an identical concept is applied to the manufacturing procedure, defined as the Critical Process Parameters (CPP), which are process parameters which have a direct impact on the material CQA, therefore requiring similar monitoring.

To ensure GMP and compliance with the quality ICH guidelines, one of the most valuable strategies that manufacturers can take advantage of is the inclusion of Process Analytical Technology (PAT) tools that provide online and continuous monitoring of CPPs. With PAT the manufacturers can base decisions on available data, and build relationships between process variability and product critical attributes which allows for better identification of CQAs and CPPs, while offering the possibility

for constant process optimization to ensure maximum yield with minimum waste. With low variability rejected batches will be diminished, cost-effectiveness will be improved and by taking a proactive stand in manufacturing the manufacturers can minimize development of manufacturing processes with deviations from regulatory guidelines which would be costly to correct in more advanced phases nearing market introduction (96)(97)(98).

The application of the QbD approach to nanomedicines again differs from regular pharmaceutical products and, in fact, a study published in 2017 by Thierry Bastogne (99) highlighted the difficulties associated with identifying CQAs for nanomedicines, with over 20 studies reviewed with great disparity between prioritized CQAs. Furthermore, it was pointed out that QbD was practically inexistent in nanomedicine development. Nevertheless, the most common CQAs of nanodrugs are size, encapsulation efficiency, zeta-potential, polydispersity index and drug release kinetics. The ultimate goal of QbD is to define a Quality Target Product Profile (QTPP), which includes the name, formulation, dosage form, route of administration, PD and PK profiles, etc., that must be achieved, via the establishment of a Design of Experiment (DoE) capable of understanding how manufacturing processes affect CQAs. The DoE is an iterative process where experiments should be made to evaluate the consequences of variability in process parameters thereby creating mathematical models of the process. Finally, applying risk-control strategies and developing a Product Life Cycle Management (PLM) plan. Scaling-up and ensuring high quality manufacturing is one of the several hurdles that affect nanomedicines in a more prominent manner compared to regular drugs.

5.2. Stability

Another crucial parameter is the stability of nanomedicines. Stability has to be considered in two different conditions, firstly under systemic circulation and secondly under storage. Stability in systemic circulation relates to physical and chemical stability, the former refers to stability against aggregation and agglomeration in plasma and cell media, while the latter refers to stability against chemical degradation. Nanomedicine instability must be controlled, otherwise, aggregation in circulation may produce embolisms, or alterations of the intended drug release profile and location may reduce or inhibit therapeutic effect (100). Under storage conditions, variables such as temperature, humidity, light exposure, pH, and others can play a role in the long-term stability of nanomedicines and must be controlled.

When nanoparticles are administered and contact with plasma, or a protein-rich medium, there is the formation of a protein layer on the nanoparticle surface labelled

as the protein corona. Recent efforts have targeted the understanding of the effects protein corona causes on the biodistribution of the drug (101)(102). Nanomedicines suffer diverse interactions with plasma components, and it is important to understand and control protein corona formation and structure, so that the consequential biological effects are predicted and engineered, avoiding unexpected results. Protein corona control and modulation can be useful for binding and targeting applications given that different biomolecular profiles interact differently with biological barriers and pathways (103)(104). The difficulty associated with biomolecular corona of NPs derives from the lack of *in-vivo* data since retrieval of administered NPs is extremely difficult (105). *In-vivo* modelling has proved insufficient for an accurate representation of *in-vivo* scenario (106). The identity of the nanomaterial and the respective protein corona is defined as the biological identity of the molecule and, combined with appropriate characterization of the physiological fluids, is critical for a precise understanding of the biological fate of nanomedicines.

5.3. IVIV Correlation

The preclinical development and assessment of novel drugs must prove not only the efficacy and effectiveness of the drug, but also ensure safety and toxicity parameters. All these features must be granted, however, it has also to be demonstrated the added value that the new drug brings compared to its competitors in the area. To determine efficacy and toxicity, the interaction of nanomedicines with biological systems needs characterization. This involves *in-vitro* and *in-vivo* studies, another challenging aspect slowing the development of nanomedicines owing both to the difficulties imposed by the lack of protocols, guidance and regulation, and to the poor *in-vitro in-vivo* correlation (IVIVC) which results in poor translation of often excellent preclinical results to clinical trials (107).

In-vitro and *in-vivo* assays exist to predict and evaluate the interaction of drugs with biological systems to obtain data regarding ADME, efficacy and safety. In addition, these assays help establish a correlation between the physicochemical properties of the nanomedicine with biocompatibility, biodistribution, bioaccumulation, clearance and toxicity. *In-vitro* models that mimic the complexity associated with human tissues, cells and diseases are hard to recreate, however, the development of animal models capable of recreating such conditions would be extremely helpful for the translation of nanomedicines. Established toxicological assays were not designed to handle nanoparticles and there is evidence that a variety of NPs can interfere with cell viability and proliferation assays (108)(109) resulting in erroneous results.

Traditional two-dimensional (2D) cytotoxicity analysis cell models lack the variety of cell populations, extracellular matrix, serum proteins and cell-to-cell interactions, nonetheless, these are still the preferred route for nanotoxicity assessment due to cost feasibility, having caused the termination of preclinical evaluations due to misleading results (110). Cells cultured on flat substrates possess significantly different shapes than those observed under *in-vivo* conditions. There is evidence that cell shape has an influence on the uptake and intracellular circulation, properties inherently highly heterogeneous among cells, hence, *in-vitro* assays performed on 2D cell cultures display misleading results (111). This matter has been poorly investigated so far. Another relevant factor for poor IVIVC associated with cell cultures used in *in-vitro* preclinical assays is the disparity in stiffness between natural tissue and culture cells. *In-vivo* different tissues vary from soft to firm, and stiffness influences cell shape and cell function, as an example, substrate stiffness is used to direct mesenchymal stem cells to different targets (112). Stiffness is another aspect unaccounted for when evaluating the preclinical outcome of novel nanomedicines, however, it is now established that new methodologies for *in-vitro* testing must be developed.

Recent effort has been placed in developing three-dimensional (3D) models such as spheroids and organoids with the intention of better mimicking natural *in-vivo* conditions. Spheroids are spherical cell aggregates which can be generated from a variety of cells. Organoids are more complex structures developed to replicate natural tissues in architecture and function (113). Table 2 shows a comparison between 2D and 3D cell cultures.

Table 1- Comparison of the main characteristics of 2D and 3D *in-vitro* cell models. Adapted from (114).

Important characteristics	2D cell culture	3D cell culture	References
Cell shape	• Cells shape is flat and elongated since the cells can only grow and expand two dimensionally • Cells grow into a monolayer on the plate	• Natural cell shape is preserved and cell growth • Cells grow into 3D aggregates/spheroids • Spheroids contain multiple layers	Costa et al., 2016; Langhans, 2018
Cell exposure to medium	• All cells in the culture receive the same amount of nutrients and growth factors from the medium in the plate • This causes more cells to be in the same stage of the cell cycle	• Nutrients does not have to be equally divided amongst all cells but can be if needed • The core cells often remain inactive since they receive less oxygen and growth factors from the medium • This process resembles the core cells in tumor cells, making it possible to mimic the behavior and structure of a tumor cell *in vivo*	Dhaliwal, 2012; Costa et al., 2016; Langhans, 2018
Cell junction	• Cell junctions are less common and less accurately represent real junctions	• Cell junctions are common and allow for cell-to-cell communication • Cells communicate through exchange ions, small molecules, and electrical currents	Pontes Soares et al., 2012; Ravi et al., 2015; Costa et al., 2016; Langhans, 2018; Lang et al., 2019
Cell differentiation	• Cell differentiation is poor	• Cells are well differentiated	Imamura et al., 2015; Costa et al., 2016; Langhans, 2018
Drug sensitivity	• Cells often have little resistance to drugs making it appear as though drugs administered to the cells were a successful treatment • Drugs are not well metabolized	• Cells often have more resistance to drug treatment • Drug metabolism is much better • Gives a more accurate representation of the drug's effects	Haisler et al., 2015; Imamura et al., 2015; Langhans, 2018
Cell proliferation	• Cells proliferate at an unnaturally rapid pace.	• Proliferation rates are realistic and can be high or low depending on technique and types of cells being studied.	Ravi et al., 2015, Langhans, 2018
Expression levels	• Gene and protein expression levels are often vastly different compared to *in vivo* models	• Gene and protein expression levels resemble levels found from cells *in vivo*	Ravi et al., 2015; Costa et al., 2016; Langhans, 2018
Cost	• For large-scale studies, it is much cheaper than using 3D culture	• Are typically more expensive than 2D cell culture techniques and require more time • 3D cell culturing reduces the differences between *in vitro* and *in vivo* drug screening, decreasing the likelihood of needing to use animal models	Ravi et al., 2015; Costa et al., 2016; Langhans, 2018
Apoptosis	• Drugs can easily induce apoptosis in cells	• Higher rates of resistance for drug-induced apoptosis	Costa et al., 2016
Response to stimuli	• Inaccurate representation of response to mechanical stimuli of cells • Cells cannot experience gravity since they are unable to expand into the third dimension	• Accurate representation of response to mechanical stimuli of cells • Cells can experience gravity giving a more accurate representation of a cell *in vivo*	Ravi et al., 2015; Costa et al., 2016
Usage and analysis	• Highly replicable and easily interpretable • Better for long-term cultures	• Can be difficult to replicate experiments • Can be difficult to interpret data	Kapałczyńska et al., 2018

With the advances in microfabrication techniques, in 2010, a new concept was presented "organ-on-a-chip" (OOAC) (115), consisting of an artificial system developed through tissue engineering and microfluidics. OOACs are usually divided in four major categories (116–119):

- Single-Organ-on-a-Chip: emulating natural organs such as heart, liver, lung, etc.
- Multi-Organ-on-a-Chip: Combining multiple tissues to evaluate systemic interferences that may occur in-vivo.
- Tumor-on-a-Chip: created to reproduce tumor microenvironments to better evaluate drug performance.
- Body-on-a-Chip: aimed at studying the human body.

These 3D cultures can be developed from a variety of cells, including genetically modified cells, and are a promising technique to improve IVIVC for nanomedicines. Figure 19 highlights the key components related to OOAC conception and engineering (120).

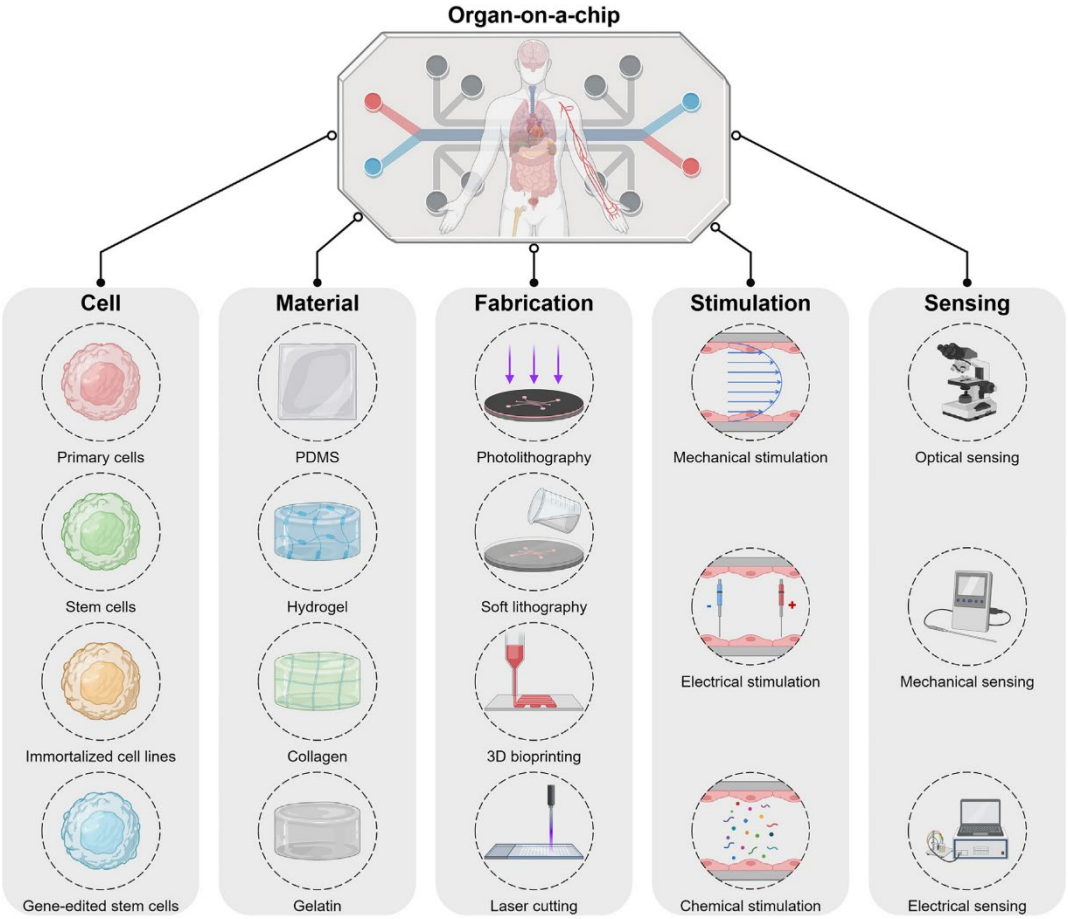

Figure 19- Key elements and steps towards development of OOAC models for drug screening. Adapted from (120).

Another aspect hindering the clinical translation of nanomedicines, which can be exploited to develop better and more efficient products is the fact that heterogeneity is present among human bodies and that affects disease development. The concept of Precision Medicine was described by the European Society of Medical Oncology (ESMO) as “the right drug, to the right patient, at the right time”, which, in other words, means that medicines could be tailored to patient specific needs and disease characteristics (121).

The same characteristics and physicochemical properties that deem nanoparticles difficult are the ones that can be exploited to develop targeted nanomedicines, including chemotherapeutics, in distant tumour sites avoiding well documented systemic side-effects of such medicines in their bulk forms (122). In this area, where a risk-benefit analysis is usually in favour of the medicine due to the severeness of the disease, the translation of nanomedicines has been poor due to poor IVIVC and significant differences in efficacy when clinical trials begin, compared to preclinical predictions. Patient-derived tumour organoids are regarded as a very promising solution to improve clinical success in clinical trials. These organoids

successfully replicate heterogeneity, genetic mutations and pathology characteristics of patient specific tumours. Sachs et al. (123) developed a biobank of breast cancer organoids developed from 150 different patients acting as a library of the disease diversity. The histopathological characteristics including hormone receptor statues and human epidermal growth factor receptor 2 (HER2) statues were preserved and catalogued. Such banks are extremely helpful for the successful *in-vitro* evaluation of effective therapies, including patient stratification (124) for increased success percentage in the translation from preclinical results to clinical outcomes. Tumour organoids are also attractive because of the possibility of mimicking the tumour microenvironment (TME) which is essential for drug screening. This includes cancer-associated fibroblasts (CAFs) and tumour vascular structures. These factors are essential to further explore the interactions between nanomedicines and tumours, understanding how nanomedicine accumulates via enhanced permeability and retention (EPR), and evaluating possible osmotic reductions due to physicochemical characteristics of the nanomaterials and specific TME variations, amongst other phenomena associated with tumour heterogeneity and nanomedicine complexity (125,126). These concepts contributed to the approval of Keytruda by the FDA as the first cancer therapy directed towards a specific patient biomarker (127).

5.4. Nanosimilars

For regulatory purposes, nanomedicines are almost always included in the pharmaceutical class of non-biological complex drugs (NBCDs) which, despite deserving a different classification from biological drugs, share a common feature. Both products cannot be fully characterized solely by chemical structure seeing that these do not present a homogenous molecular structure, thereby requiring a specific and varied set of physicochemical properties and assays for similarity with a reference product to be validated [108].

Determining the pharmaceutical equivalence and bioequivalence is not enough to demonstrate the equivalence between a nanosimilar and a reference nanomedicine. This poses a problem because the regulation approved for generic medicines is based in the determination of the aforementioned parameters. The performance of nanomedicines can be different depending on the administration route and mechanisms of cellular uptake in target tissues, hence the established methods for validating bioequivalence might not reflect the performance of nanomedicines (128)(129).

Despite the complexity of the nanomedicines, in Europe they can be approved via the non-centralized EMA procedure applied to pharmaceutical drug products. From

2015 onwards, EMA changed its posture towards approval of nanosimilars and adopted a “totality-of-evidence” approach following Article 10(3) of the Directive 2001/83/EC (130). Additionally, reflection papers specifically referring to liposomal products and iron-carbohydrate complexes and their nanosimilars have been released by EMA to guide the regulatory process from a totality-of-evidence perspective (130–132). Considering, for instance, IV nano-colloidal iron-carbohydrate complexes, the demonstration of similarity implies the demonstration of bioequivalence and plasma iron concentrations. Iron plasma concentration is strongly affected by the stability and physicochemical properties of the product and, consequently, so are the toxicological and pharmacological properties of the product. A full physicochemical characterization profile must also be submitted to ensure quality. To approve a nanosimilar safely, data from quality, non-clinical studies and PD and PK assays are mandatory, with the possibility of additional clinical studies. Nonetheless, examples of clinical success exist in the market as proved by Venofer®, which has been widely used to treat iron-deficiency since 1949 (133).

In 2012 the FDA was forced to investigate nanosimilars due to a severe shortage of doxorubicin hydrochloride liposome injection. At the time, the importation of a replacement generic, Lipodox, which is a nanosimilar liposome formulation was approved. Both Doxil and Lipodox contain the same amount of API and the same liposomal composition and, having established bioequivalence, approval was granted (134,135). On the contrary, in 2011, the same medicine had its marketing application withdrawn in Europe because EMA declared that the presented bioequivalence and *in-vivo* studies were insufficient to support similarity. As mentioned, another great example reinforcing the difficulties associated with nanosimilars are IV iron-carbohydrate complexes. These are composed of a polynuclear iron(III)-oxyhydroxide core shell stabilized using a carbohydrate shell. There is a plethora of commercialized options in different markets, and it has been established that the *in-vivo* characteristics of the product depend on the nanoparticle size, shell chemistry and manufacturing process (136). The stability of the final formulation dictates the biodistribution, bioavailability, clearance, drug release profile, safety, dosage, etc. As a result, there is evidence that nanosimilars approved via the common methods differ from the respective reference materials, with preclinical studies revealing differences in terms of inflammatory responses in the liver, heart and kidneys. The similar iron sucrose drugs possess differences from the reference drugs in terms of particle size, particle size distribution and visual appearance (137–139).

5.5. Amphotericin B: A Case of Success.

Despite the significant challenges described in this section, there are some success cases to report. One such example is the case of Amphotericin B (AB), which stands as a primary choice for the initial treatment of suspected or confirmed invasive fungal infections (IFIs), particularly in patients who are critically ill. One of the main disadvantages when conventionally formulated as AB deoxycholate is the number of side effects, such as fever, nausea, vomiting, chills, rigours, anaemia and, more importantly, nephrotoxicity (140,141).

Given the amount and severity of adverse effects efforts were placed in developing new AB formulations and, between 1995 and 1997 three novel forms were approved by the FDA, AB lipid complex (ABLC), AB colloidal dispersion (ABCD) and liposomal AB (LAB). These alternatives presented better safety profiles and fewer adverse effects, however, the elevated manufacturing costs have hindered their implementation as the common therapeutic approach. A low-cost alternative consists of an infusion of conventional AB in intralipid form, a lipidic emulsion for parenteral nutrition (142).

The systematic review performed by Tonin et al. (143) Carried a network meta-analysis to withdraw specific conclusions regarding the efficacy and safety of the different AB formulations. The analysis took into consideration 25 different trials, comprising 2996 patients treated with a minimum of 1 dose of any AB formulation. Factors considered for evaluation and ranking were efficacy as the cure, deaths, fever, chills, nephrotoxicity and discontinuation. From the listed categories, AB Intralipid ranked first in all apart from efficacy as cure and deaths, albeit ranking above Conventional AB for all categories.

Even though the work is not a direct comparison between formulations, the analysis was conducted following the good practices and standards in systematic meta-analysis and proves that nanomedicines can present benefits when compared to conventional forms. Intralipid is approved for use in Europe and the USA since 1975, before any attention was paid to the safety of nanomedicines. This leaves an opening towards a simpler approach regarding nanomedicines, in the way that existing regulation is enough to screen drugs that are effective and safe to introduce in the market, and that nanomedicines don’t necessarily require a complete rewriting and restructuring of the regulatory framework already developed for conventional pharmaceutical products.

6. Regulatory Approach for Nanomedicines

The objective of this chapter is to correlate the information gathered in chapters 3 and 4, regarding the regulatory procedure that is applied for human-use drug products and the efforts that regulatory agencies and governments have directed towards developing the regulatory framework of nanomaterials in response to the technological development that has led to the implementation of nanotechnology in various healthcare sectors, and the findings of chapter 5, which highlight the biggest difficulties in getting a nanomedicine through the entire preclinical and clinical development with success, culminating in a grant of market approval.

From the information reviewed it is safe to conclude that the primary difficulty associated with nanomedicines is the significant lack of data available to manufacturers derived from the immense variability that these materials entail. In this sense, the methodology that the FDA and EMA are leaning on to advance regulation is correct. The development of technical centres such as the Nanotechnology Characterization Laboratory (NCL) and the Centre for Drug Evaluation and Research (CDER) in the USA, or the European Nano-Characterisation Laboratory (EU-NCL) in Europe are essential to carry mass characterization of nanomaterials for healthcare application, while also researching future scientifical procedures and avenues that can perform a better job of evaluating the characteristics of nanomedicines. The creation of specialty taskforces is also imperative, to promote cooperation between agencies and experts, so that relevant aspects are discussed and planning for future actions, programmes, deliverables, fundings or any other relevant activities are established. Regarding these aspects the actions of both EMA and the FDA have been extensive and in continuous growth.

Nonetheless, after an extensive search and literature review, the EU stands out in the availability, presentation, transparency, and number of concrete reports when compared to the USA. One such example would be NanoReg II, a very extensive project with a significant amount of disclosed information, including reports on the execution of the project and the proposed deliverables. From this project meaningful conclusions towards necessary regulatory actions are withdrawn and, more importantly, the development of the NANOREG toolbox and the SIA framework are very specific, useful and valuable tools for manufacturers and regulatory agencies. The creation of trusted environments and the involvement of regulatory activities early and more frequently during initial development and manufacturing phases is also critical to prevent failures while scaling-up the process.

Overall, the progress and the direction of the regulatory approach for nanomedicines are positive, and an approach on a case-by-case basis following the scrutiny of safe, tested, and long-term established existent regulations is a cautious and righteous approach. Nevertheless, whenever significant information is available amendments and updates should be issued, including, if necessary, updates to ICH guidelines on quality, non-clinical reports and clinical reports.

It is therefore more important to overcome the challenges identified in Chapter 5, with help from regulatory agencies, than to develop an entirely new regulatory framework for nanomedicines, which, given the variety of materials and applications would be extremely difficult. In the following sections an analysis is performed on the role that regulatory agencies can have in developing guidelines and documents to overcome the challenges identified.

6.1. QbD and CQAs

Manufacturing is responsible for ensuring the quality and safety of nanomedicines. As discussed, the scale-up process from the laboratory and research scale towards the manufacturing scale of the final product is problematic due to the sensitivity of nanomaterials to minor differences in processes, which undoubtedly exist when going from the lab to the manufacturing plant. It is essential to implement QbD principles early in the development phase.

For QbD principles to work, including the implementation of in-process controls, the priority should be the identification of critical quality attributes of the materials being developed. Those CQAs must be monitored and evaluated not only during manufacturing, but also when any modifications to manufacturing processes are performed. Identification and evaluation of CQAs relies on existence of effective analytical characterization procedures, procedures that should be listed in guidance documents and regulation. Figure 20 highlights physicochemical and biopharmaceutical effects caused by variation of nanomedicine formulation parameters.

The creation of a globally recognized list of CQAs accompanied by a dedicated guideline would definitely be useful to ensure the submission of high-quality standardized data for nanomedicines in development and Clinical Trials, expediting access to nanotechnology health products, as detailed in Fig. 21. The major obstacle in the creation of such tools is the very rapid development of technologies on the manufacturers' side, which is difficult for regulators to stay on top. Conjugated with the speed of development is the increase in complexity, therefore, regulators must adopt a "fit-for-purpose" system, which balances the compromise between innovation and

rigorous efficacy and safety standards. This system should also involve cooperation and integration.

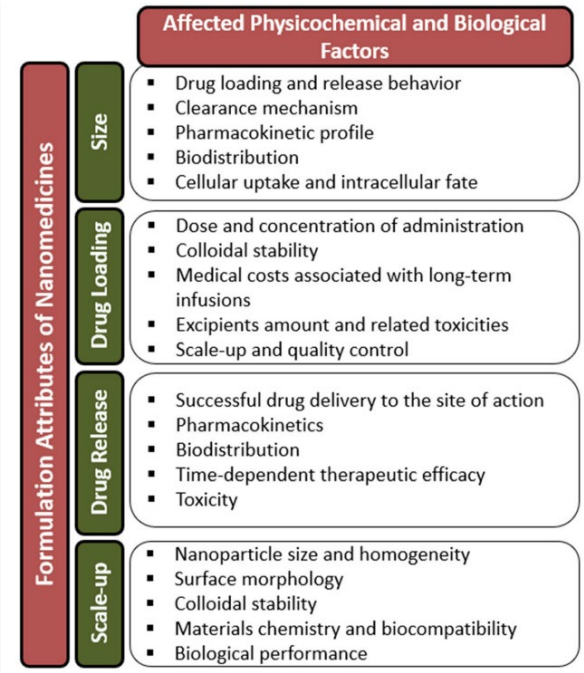

Figure 20- Physicochemical and biopharmaceutical effects caused by variation of nanomedicine formulation parameters. Adapted from (144).

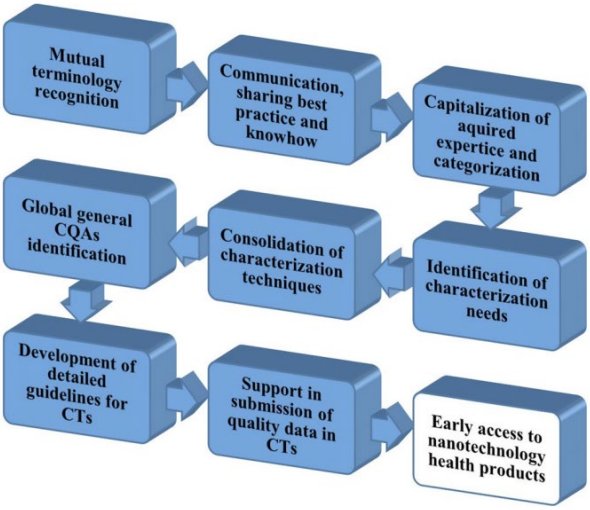

Figure 21- Pathway towards the creation of a harmonized CQA for expedited access to nanomedicine technologies. Adapted from (145).

6.2. Preclinical Development Considerations

Besides the necessary implementations to ensure quality materials and pharmaceuticals, it is important for manufacturers to obtain guidance in the development of appropriate *in-vitro* studies that translate well *in-vivo* assays and, consequently, to clinical trials in humans. Poor IVIVC has been identified as one of the

most common causes of failure in the clinical translation of nanomedicines, therefore, it is also urgent to act in this field. In this field researchers must develop *in-vitro* models that accurately represent the physiological media and cell cultures where the nanomedicine is expected to operate, such as the 3D cell models indicated in Chapter 5. The design of preclinical trials using innovative models should be reported to regulatory agencies, as well as justification and validation reports, so that guidelines can be updated and issued. Again, centralization and harmonization of procedures are very important. If regulators have early access to these developments and standard protocols are established future decisions are expedited and better counseling can be provided.

In addition, the bio-interactions between nanomedicines and biological systems must be evaluated, and regulatory agents are urged to develop standard mandatory tests to assess and characterize these interactions. An example is the formation of biomolecular corona once nanoparticles contact a protein-rich environment such as plasma. Characterization of biomolecular corona is helpful not only in understanding the effects it causes on the ADME profile of the drug, but also to gain the ability of previously engineering what type of biomolecular corona is formed, so that the effects it causes are predicted instead of random.

Ensuring the aforementioned factors will dramatically increase the confidence level of manufacturers when transitioning from *in-vitro* to *in-vivo* conditions, as well as when clinical trials are initiated. Failure in Phase I clinical trials has tremendous costs for manufacturers, given that the further the stage of development failure occurs, the more economic and time investment has been performed. Increasing the confidence level is urgent to increase willingness of manufacturers to bet in novel technologies. The ability to consult updated guidelines and databases with remarks from regulators, experts, and other manufacturers is again essential.

Another particularity to be explored is patient stratification and personalized medicines, an extremely unexplored area both from regulators and manufacturers, but with good upsides and potential.

6.3. Nanosimilars

Nanosimilars can be approved via three abbreviated and expedited pathways, generic, hybrid and biosimilar. These procedures approve “follow-on” medicines, which consist of medicines which are granted approval based on pharmaceutical equivalency to a reference drug product (146). The FDA has specific guidelines for the evaluation of complex generics, and according to 505(j) NDA such products can be approved based on bioequivalence (147). EMA does not have specific regulatory provisions for

complex follow-on medicines, such as nanomedicines. Since 2015 EMA has shifted towards a totality-of-evidence approach for the approval of nanosimilars via the hybrid pathway, despite not having any specific guidelines for this matter apart from intravenous iron-based nano-colloidal products and intravenous liposomal products.

The FDA guidelines have been criticized for not being adequate for the complexity of nanomedicines, and the adoption of a different approach was suggested to ensure safe approval of quality medicines (148). Differences in manufacturing will undoubtedly lead to differences pharmacokinetic and pharmacodynamic properties caused by physicochemical alterations which are hard to assess, control and predict. One possibility for regulators would be to proceed with a stepwise approach. In this system, a thorough functional and structural characterization constitute the first step, and from the conclusions it would then be determined which additional information packages should be evaluated to prove nanosimilarity, such as PD/PK studies, *in-vivo* assays and, if necessary, clinical trials. EMA has the possibility of approving generics and follow-on medicines via the centralized system using the hybrid route, i.e., an application which involves using both a reference medicine and new data from additional relevant tests given that differences are verified from the reference medicine and bioequivalence studies are not sufficient to assure similarity. An important issue that remains unregulated and needs addressing is the interchangeability and substitution of nanomedicines.

Nanomedicine follow-on products should not be evaluated solely on bioequivalence studies, and approaches which involve thorough characterization are necessary. Therefore, nanomedicines must be grouped into a class where approval based on reference medicines requires significant data to assure similarity and obtain approval via centralized procedures prepared to deal with the complexity of these materials.

7. Conclusion

Regulation of pharmaceutical products for human use is an extremely complex area that has been in constant development for years. Nanomedicines introduce a high degree of complexity for which the existing regulatory framework is not prepared, despite huge efforts which have been conducted by the FDA and EMA.

A literature review has proven that regulatory agencies and governments, more particularly, the United States and European Union stakeholders, have been focusing greatly on launching initiatives and programs to adapt the regulatory frameworks to the characteristics of nanomedicines, however, there is still a long way to go, and that nanomedicine regulation will require a proactive attitude from regulators. From the manufacturing and development standpoint, innovation is happening at great speed with new materials, procedures and technologies which are unknown to regulators and unstudied. Understanding how these innovations interact with the human body is the responsibility of manufacturers, but also of regulatory agencies to overview and ensure that the analytical procedures used are validated and valid conclusions are reached. In this sense, regulation of nanomedicines requires all the stakeholders to be involved from very early development phases, to ensure that clinical trials are reached with a high degree of confidence in the quality, effectiveness and safety of the product being developed.

Despite the huge potential that nanomedicines have shown in initial laboratory tests, often the results diverge when advanced *in-vivo* assays begin. This is due to the complexity involved in the manufacturing processes of these drug products and the unknown interactions of nanomaterials with biological systems, which are characterized by peculiar effects such as the biomolecular corona formation, which can significantly modify the ADME profile predicted and intended for nanomedicines.
From a regulatory perspective, it is deemed more important to develop the knowledge surrounding nanomedicine development and nano-bio-interactions, with the introduction of databases, policies of shared information, the introduction of harmonized CQAs and implementation of QbD policies in manufacturing, development of innovative and more realistic *in-vitro* assays, to ensure that the products being developed have high quality and performance, with very low margin for error when advancing to further steps in the clinical development process.

Implementing a completely novel set of regulations specifically for nanomedicines is not short of impossible, due to the variability of materials, technologies, and applications. In this sense, the evaluation of nanomedicines in a case-by-case approach is considered the correct way, and the more pressing issue is

to resolve the low clinical translation of nanomedicines. Good manufacturing, non-clinical assays and clinical evaluations will ensure compliance of the products with existing regulations which have been developed for a long time and dealt with hundreds, if not thousands, of applications. This regulation is robust, at a very mature stage, and has proven over time that ensures that only safe, effective and high-quality products reach the market.

On the other hand, if regulators are more involved in the development phases and the risks identified are minimized in the early phases, the confidence level in these types of medicines will increase, which will encourage manufacturers to bet on these technologies more willingly, ensuring more research and funding.

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