Universidade de Lisboa

Faculdade de Farmácia

DISSERTATION

GERIATRIC MEDICINES DEVELOPMENT: IMPROVING REGULATORY STRATEGIES TOWARDS ESTABLISHING AN EVIDENCE-BASED BENEFIT-RISK BALANCE IN THE GERIATRIC POPULATION

Sara Cristina Traguedo Alfenim

Dissertation Report supervised by Assistant Professor at Faculty of Pharmacy, University of Lisbon, PharmD MSc PhD, João Pedro Fidalgo Rocha and co-supervised by Full Professor at Faculty of Pharmacy, University of Lisbon,PharmD MSc PhD MPH, Bruno Miguel Nogueira Sepodes

Regulation and Evaluation of Medicines and Health Products

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Abstract

The worldwide growing geriatric population, with high incidence in Europe, raises a sense of responsibility on geriatric patient representation in medicines development, considering younger adults' studies data cannot be directly transposed to the elderly. In this study, a detailed understanding of aging and age-related diseases is presented for the characterization of the geriatric patient to further make the connection with the requirements needed for an effective geriatric patient-centric medicinal products development. The current regulatory that supports medicinal products marketing approval and clinical trials execution as well as the interaction between clinical trials environment and the geriatric subjects are deeply analyzed. A set of regulatory proposals is presented for the improvement of evidence-based geriatric medicines development.

Key words: geriatric; elderly; clinical trial; regulatory; evidence-based; patient-centric; medicines

Resumo

O crescimento da população geriátrica mundial, com elevada incidência na Europa, cria um sentido de responsabilidade na participação dos doentes geriátricos no desenvolvimento de medicamentos, considerando que os dados obtidos em estudos com adultos mais jovens não podem ser directamente extrapolados para os idosos. Nesta dissertação, é apresentada uma explicação detalhada sobre o envelhecimento e as doenças relacionadas com a idade de forma a permitir caracterizar o doente geriátrico e, posteriormente, fazer a ligação com os requisitos necessários a um eficaz desenvolvimento de medicamentos centrado no paciente geriátrico. A legislação que, actualmente, suporta a aprovação de medicamentos para comercialização e a execução de ensaios clínicos, assim como a interacção entre o ambiente dos ensaios clínicos e o sujeito geriátrico são objecto de uma análise profunda. É proposto um conjunto de medidas regulamentares para a melhoria do desenvolvimento de medicamentos geriátricos baseado em evidências.

Palavras-chave: geriátrico; idoso; ensaio clínico; regulamentar; baseado na evidência; centrado no paciente; medicamento

Acknowledgements

I would like to dedicate this dissertation to my dear father António Alfenim and to memory of my mother Joana Alfenim, for always supporting me in my life goals, and to my son Miguel Rabeca, for his patience and understanding of his mother's dedication to the study and writing of this text.

To Professor João Rocha I give my thanks for having accepted to give me his sharp guidance on this challenge and the fruitful result from our interactions.

Abbreviations

ABCs	Age-associated B cells
AGE	Advanced glycation end- products
AMPK	AMP-activated protein kinase
APOE	Apolipoprotein E
AUF1	Adenylate-uridylate-rich element RNA-binding Protein 1
CGI	CpG island
CHMP	Committee for Human Medicinal Products
CMV	Cytomegalovirus
COPD	Chronic obstructive pulmonary disease
CpG	Cytosine-Guanine dinucleotide site
CR	Caloric restriction
CRP	C-reactive protein
CTIS	Clinical Trials Information System
DCR2	Expression of decoy death receptor 2
DEC1	Embryonic chondrocyte- expressed 1
EMA	European Medicines Agency
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
FDA	Food and Drug Administration
FGF2	Fibroblast growth factor 2
GEG	Geriatric Expert Group
GIP	Geriatric Investigation Plan
GFR/ eGFR	Glomerular filtration rate/ estimated glomerular filtration rate
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
GWAS	Genome-wide association studies
HLA	Human leukocyte antigen
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
ICMRA	International Coalition of Medicines Regulatory Authorities
IEC	Independent Ethics Committee
IFN	Interferon
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth factor-binding proteins
IL	Interleukin
ILS or IIS	Insulin/insulin-like growth factor signaling

IMP	Investigational medicinal product
IND	Investigational new drug application
irAE	Immune-related adverse effects
IRB	Institutional Review Boards
irRC	Immune-related response criteria
IRF	Interferon regulatory factor
LSEC	Liver endothelial sinusoidal cell
MA	Marketing authorization for medicinal products
MacCAT-CR	MacArthur Competence Assessment Tool for Clinical Research
MHC	Major histocompatibility complex
MHLW	Ministry of Health, Labour, and Welfare
miRNA	microRNA
MMP	Matrix metalloproteinases
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
NAD+	Nicotinamide adenine dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NF-κB	Nuclear factor kappa B
NK	Natural killer
OIS	Oncogene induced senescence
PBPK	Physiologically based pharmacokinetic
PD	Pharmacodynamic
PIP	Pediatric Investigation Plan
PK	Pharmacokinetic
PMDA	Pharmaceutical and Medical Devices Agency
PSA	Polysaccharide A
RAGE	Receptor for advanced glycation end- products
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SA-β-gal	Senescence- associated β- galactosidase
SASP	Senescence-associated secretory phenotype
SCFA	Short-chain fatty acids
SPPB	Short Physical Performance Battery
STAC	Sirtuin activating compounds
STAT	Signal transducer and activator of transcription
TCR	T cell antigen repertoire

Th	T-helper cells
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor-alpha
TOR	Target of Rapamycin
Treg	Regulatory T cells
UBACC	University of California Brief Assessment of Capacity to Consent
USA	United States of America
WHO	World Health Organization

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1. Characterization of the geriatric patient

Aging is biologically associated with a progressive decline in physiological homeostasis resulting in impaired function and increased susceptibility to death. [1] In the aging world, natural selection is not able to maintain evolutionary fitness at older ages [2] and, as a consequence, the saved population is exposed to an elevated risk for aging-related diseases [3], like cancer, cardiovascular diseases, neurodegenerative diseases [4] [2] [5], osteoarthritis and macular degeneration [5]. Age itself becomes a primary risk factor for the length and quality of life [3], as for the development of the chronic fatal diseases [4] [2] [1].

Societies are rapidly aging with an increasing healthcare burden related to morbidity and cost of age-related diseases. [5] The modern medicine focus on the chronic fatal aged-related diseases originates a competing risk between them. [3] Decreasing the death risk from one disease will signalize or increase the risk of another one. [3] Also, current medical care is highly segmented and organ/disease based ignoring the fact that age and the aging process are the strongest risk factor for each of these diseases. [5] The healthy and disease free lifespan (known as healthspan) [4] has not increased as much as overall life expectancy [4] [2] with a 16-20% average of late-life time spent with morbidity. [4]

The advances in biology of aging has been showing that part of aging is controlled by genes and biochemical pathways conserved in evolution which leads to the possibility of effective interventions development to delay or decrease the age-related pathologies with extension of healthy lifespan. [1] Therefore, for people older than 65 years, the primary goal of medicine should now shift to the extension and improvement of healthspan in order to delay and reduce the period of the lifespan when frailty and disability increase substantially [3], in other words, to achieve the compression and reversal of late-life morbidity [4] [2]. This is known as the *first health revolution* and it is seen as an effective primary prevention tool in public health methods. [3] It originated a new science - geroscience - which studies the aging biology and develops specific clinical trials. [3] The target of the cross-disciplinary field geroscience is to anticipate the aging pathways in order to protect against the age-related diseases. [5] [1] For the understanding of the link between aging mechanisms and the chronic disease susceptibility, seven areas have been identified as the main pillars for the biology of aging research: inflammation, adaptation to stress, epigenetics, metabolism, macromolecular damage, proteostasis and stem cells. [1]

Individuals who live beyond 100 years show progressively greater compression of late-life morbidity meaning that a healthy end of life is physiologically possible. [4] [2] The development of future interventions for the delay of the aging process and reduction of morbidity needs a better understanding of aging mechanisms at different tissues and at different stages of life - with consequences to the development of age-related diseases - for

which animal models studies are useful as a first approach to further better target the human studies. [4] Although similarity in responses between animal studies and interventions in humans needs to be proven, a major conclusion from aging in model organisms is that interventions to delay and/or prevent age-related diseases are a realistic possibility. [4]

The early studies in aging mechanisms show that the number of genes that control aging process is larger than expected (higher level of plasticity in the aging process), and that those genes are conserved across wide evolutionary distances, sensitive to interventions in order to extend healthspan and lifespan. [5] These preventative interventions can happen at the lifestyle level (specific diet-management of protein and carbohydrate intake, physical activity, cognitive training, vascular risk management, caloric restriction, intermittent fasting), at drugs level or, even, young microbiome transfer and human umbilical cords blood/plasma/plasma proteins transfer and genetics. [4]

1.1. Evolution of the geriatric population

Lifespan is a worldwide individual record for longevity [3] which maximum figure is assumed to be a stable characteristic of a species [6], different from life expectancy which is a population-based metric using statistical calculations. [3]

Lifespan stands currently on 122 years (age at death of Jeanne Calment, currently the oldest documented human individual who ever lived [6]) and it is not expected to occur an increase during the current century. [3] A controversial debate is ongoing about the existence of a fixed maximum lifespan for humans. A study of individual survival trajectories of Italian inhabitants aged 105 years and older during 6 years strongly suggested that longevity is continuing to increase over time and that a limit, if existing, has not been achieved. [7] On the other hand, a study based on Human Mortality Database shows a plateau after 1995 in the improvement of survival (maximum reported age at death), suggesting that human lifespan may have a natural limit (although without a definite conclusion). [6] At the moment, it is unclear if a limit to human lifespan is to be seen. [2]

As for the life expectancy, the increase in life expectancy at birth in developed nations has reached around 30 years since the late 19th century (at the time, ranged between 45 and 50 years), by implementation of public health with measures of sanitation and clean water [3], hygiene, immunization and antibiotics [4] that resulted in the reduction of childhood mortality due to infectious diseases and later by the declining of death rates in older ages. [3] [4] Once childhood diseases reduction is achieved the only other way to increase life expectancy is by medical advances and improvement of lifestyles with impact in older people survival. [3] The age around 85 years (man and woman combined) has been achieved in many parts of the world as the point of diminishing returns on life expectancy and it has been approaching the lifespan. [3] Due to this, some assume that the goal of life extension has largely been achieved

[3] while for others the evidences indicate that the survival rates in elderly and the mean life expectancy are projected to continue to increase [4] [2], despite a controversial limit of 115 years has been determined [2].

The importance of improving the geriatric health management and the diseases treatment is stressed by the demographic evolution observed since 1950 and the forecast towards 2050, year 2019 data by United Nations (Figure 1.1, Figure 1.2, Figure 1.3, Figure 1.4, Table 1.1, Table 1.2), which clearly shows the population rapid aging.

For societies worldwide, the aging human populations has arose health challenges with the major burden falling on older people at the same time that birth rates decline. [2] As a consequence, there is an increase in the ratio dependent to independent members of society which originates major economic and social problems. [2]

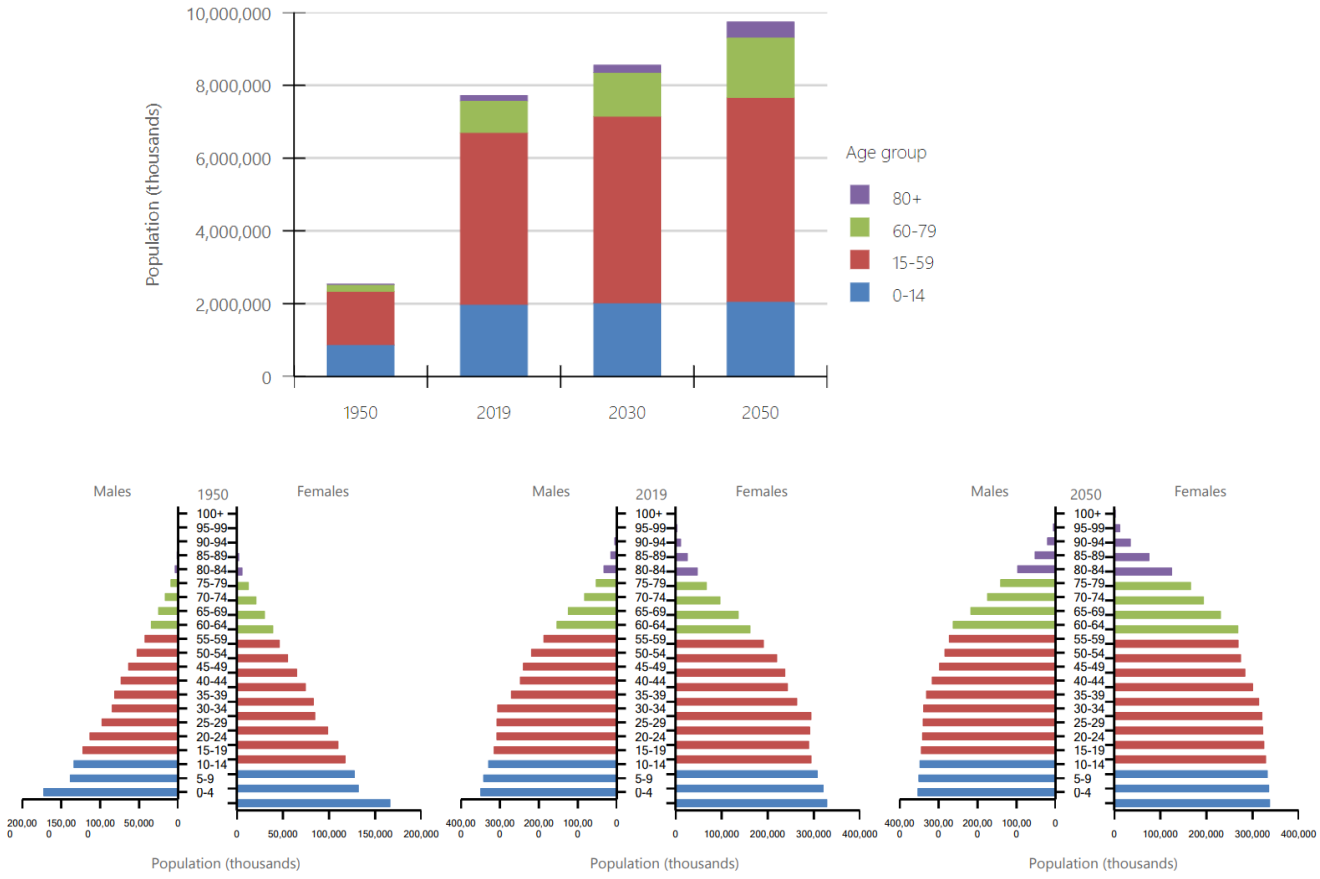

Figure 1.1 World profile of aging 2019, by age group (thousands) [8]

The total world population is expected to increase around 5 times in the frame of 100 years (1950- 2050) with a drastic change in the shape of the age populational pyramid due to the population increase in ages older than 40 years. It shows a decrease in the potential support ratio (ages 20-64/ ages 65+) from 10.1 (year 1950) to 3.5 (year 2050) [8] - around 2.9 times. This ratio indicates a trend for existing less active age people to support the elderly.

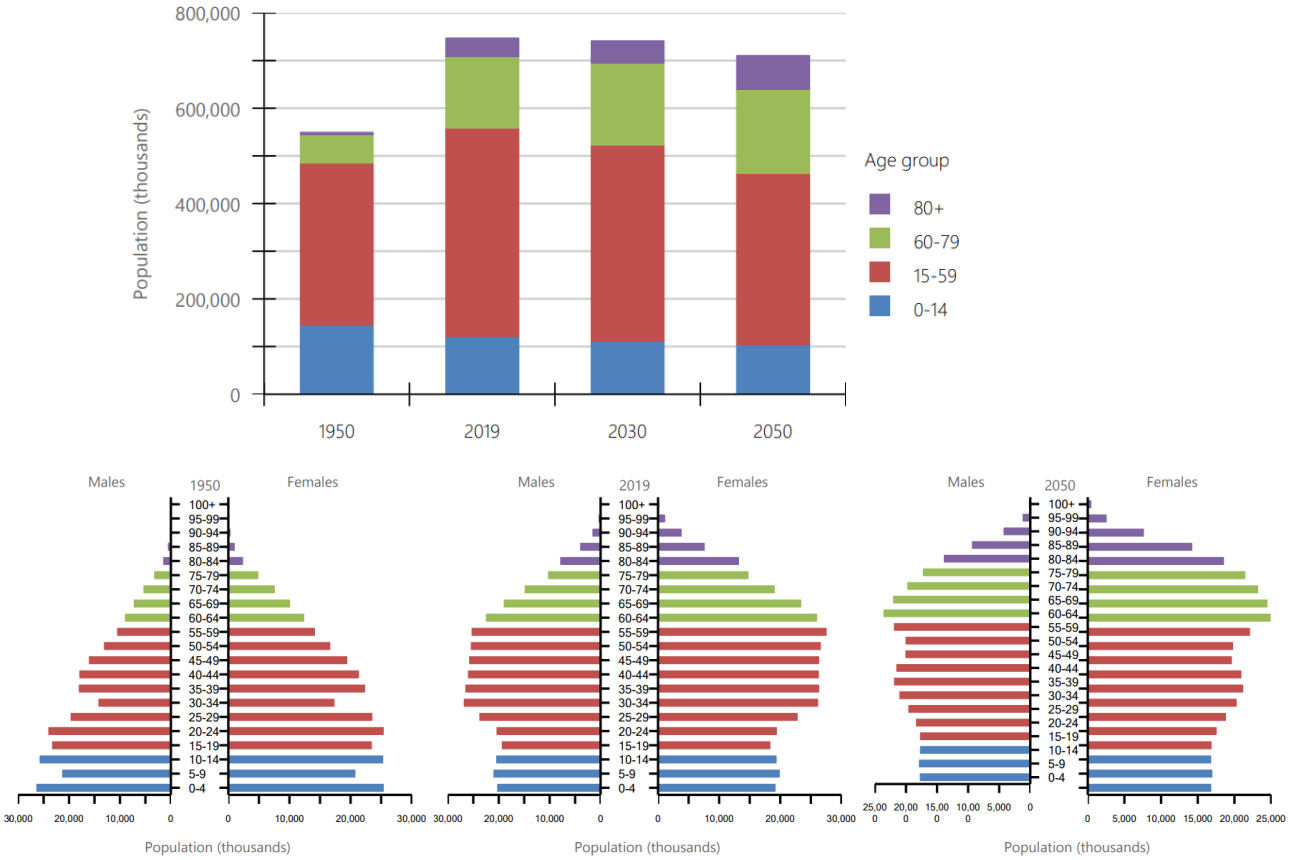

Figure 1.2 Europe profile of aging 2019, by age group (thousands) [8]

In Europe, the expected total population increase for the same frame of 100 years (1950-2050) is more modest (around 0.3 times) with a trend to population decrease from 2019 onwards. The change in the shape of the European age populational pyramid shows a reduction of population in age groups below 60 years and a huge increase of population aged above 60 years. Comparing with worldwide ratio, there is a higher decrease in the potential support ratio (ages 20-64/ages 65+) from 7.2 (year 1950) to 1.9 (year 2050) [8] - around 3.8 times. This ratio indicates a trend for existing less active age people to support the elderly.

Age	1950	2019	2030	2050
0-14	34.3	25.6	23.6	21.1
15-59	57.7	61.2	59.9	57.5
60+	8.0	13.2	16.5	21.4
65+	5.1	9.1	11.7	15.9
80+	0.6	1.9	2.4	4.4

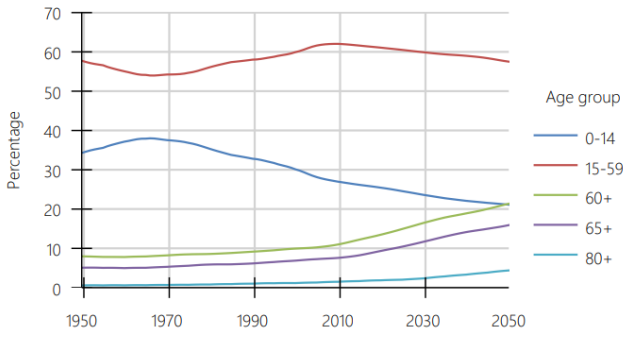

Figure 1.3 World profile of aging 2019, by age group (percentage) [8]

It is expected to have around 16% of world population aged 65+ by 2050, which is double than year 2019 for the same age fraction.

Age	1950	2019	2030	2050
0-14	26.3	16.1	14.9	14.6
15-59	61.8	58.7	55.6	50.5
60+	11.8	25.3	29.5	35.0
65+	8.0	18.8	23.0	28.1
80+	1.0	5.3	6.3	10.1

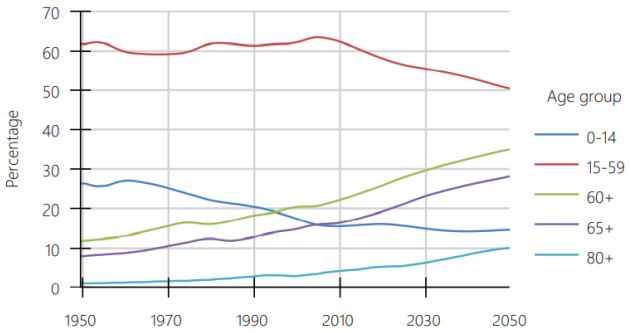

Figure 1.4 Europe profile of aging 2019, by age group (percentage) [8]

For Europe, it is expected around 28% of population will be aged 65 by year 2050, which is 10% additional to year 2019 for the same age fraction.

Table 1.1 World profile of aging 2019, life expectancy (years) [8]

	1950-1955		1980-1985		2010-2015		2045-2050	
Age	Males	Females	Males	Females	Males	Females	Males	Females
0 (birth)	45.5	48.5	59.8	64.4	68.5	73.3	74.5	79.1
60	13.0	15.1	15.8	18.8	18.6	21.7	21.5	24.3
65	10.4	12.1	12.6	15.3	15.1	17.8	17.8	20.2
80	4.8	5.5	5.7	6.9	7.1	8.5	8.7	10.0

At worldwide level, it is expected to increase around 30 years in the life expectancy at birth for the considered time frame (1950- 2050).

For all geriatric age groups, the remaining life expectancy shows an increasing trend.

Table 1.2 Europe profile of aging 2019, life expectancy (years) [8]

	1950-1955		1980-1985		2010-2015		2045-2050	
Age	Males	Females	Males	Females	Males	Females	Males	Females
0 (birth)	61.0	66.2	67.6	75.6	73.6	80.7	80.0	85.4
60	15.5	17.8	16.2	20.4	19.8	23.9	23.8	27.3
65	12.3	14.1	13.0	16.5	16.4	19.7	20.0	22.9
80	5.3	5.9	5.8	7.1	7.6	9.0	9.8	11.0

At Europe level, it is expected to increase around 19 years in the life expectancy at birth for the considered time frame. When compared to the worldwide figures, it is observed that European life expectancy at birth starts at a higher figure (66 years Europe vs 48 years worldwide, for females at year 1950) and ends, also, at higher figures (85 years Europe vs 79 years worldwide, for females at year 2050).

For all geriatric age groups, the remaining life expectancy shows an increasing trend.

1.2. Theories of aging

Aging can be defined as changes that occur in an organism lifespan at a rate that varies widely. [9] While some of these changes are innocuous because they do not affect individual's viability - for example, wrinkles - other changes originate increased risk of disease, disability or death and, for a distinction, the later can be designated with a more precise term - senescence. [9] These changes take place at cellular, molecular, tissues and organ systems levels. [9]

The classic theories of aging are mainly related to short-lived species in which the mortality increases and the fertility decreases after maturity but the pattern of senescence is very diverse, [2] for example, there are species with weak or no signs of aging with advancing age and there are also species with negative senescence (physiological improvement with age) [2] [10]. Nevertheless, it might happen that the aging is not detectable because the studied individuals were not old enough. [2] Examples of these species are the freshwater polyp *Hydra* (potentially immortal, the fertility and survival do not decrease with age) [2] [10], the bristlecone pine *Pinus longeava* (live for thousand years), the naked mole-rat *Heterocephalus glaber* (mortality does not increase with age) and the bowhead whale *Balaena mysticetus* (longest lived mammal, with maximum estimated lifespan of 211 years). [2] Other examples show that natural selection does not always decline with age, often due to starting reproduction before they have finished growing (some invertebrates and fish, for example), or by protection of germline cells until adulthood keeping high regenerative potential (some higher plants). [2]

There are hundreds of theories trying to explain the cause of aging which can be grouped into two main categories (among others possibilities): program theories and error theories (a combined group with a mix of elements from both theories also exists). [9] Neither one single cause nor a universal cause of aging valid for all living organisms can be assumed. [9]

1.2.1. Program theories of aging

The program theories are adaptive aging theories describing evolutionary benefits from the existence of a limited lifespan. [9] The deliberated deterioration of organisms with elimination of pos-reproductive age individuals would avoid the overpopulation and related resources competition and promote adaptation through the succession of generations. [9] Also, this process could be modulated by aging genes and the existing studies with gene mutations in model organisms show results in lifespan extension but no results in aging cancellation. [9] Same results came from studies based on endocrine system implying growth hormone (GH) and insulin-like growth factor-1 (IGF-1) which could be attributed to an indirect consequence of the developmental program of organisms. [9] The program theories- pre-determination of aging- are challenged by the fact that numerous species have significant different lifespan under the same conditions and by the fact that lifespan can be prolonged or shortened under

specific conditions. [9] Also, a number of studies suggest that senescence might not be universal, so, the evolution of aging could not be a programmed process. [2]

1.2.2. Damage theories of aging

Some damage theories, defending the non-programmed cause of aging, present it as the absence of natural selection at the post-reproductive stage of life. [9] It is thought that the effect of natural selection in human aging has a larger impact earlier in life, with consequences on the survival and reproduction than in late life period. [2] This is due to the influence of extrinsic hazards - caused by environmental - (example, diseases or accidents) causing the decrease of population in adult ages independently of the genetic variants that could impact the fitness at later ages (for bearers and non-bearers). [2] According to population genetic theory of aging, this influence of extrinsic factors in natural selection leads to aging mechanisms of mutation accumulation (accumulation of deleterious mutations at higher frequency in late life period, due to weakened force of natural selection) [2] and/or antagonistic pleiotropy (mutations or alleles with positive effects early in life are favored by selection despite the negative effect the same genetic variants will have late in life, when selection action is weaker) [2] [10].

One popular damage theory is the oxidative damage by reactive oxygen species (ROS) when present in high levels, generated during metabolism and causing accumulation of DNA, protein and lipid damage [9]:

- Mitochondria are the major producer of ROS in mammalian cells which leads mitochondrial DNA (mtDNA) to be very susceptible to mutation causing cellular damage and being already identified as a hallmark for several human pathologies and aging [9];
- ROS are active intermediate in DNA methylation and it signals could be a cause for DNA hypomethylation characteristic of the aging process (more data needed) [9];
- Irreversible oxidative damage to proteins is subject to a proteolytic degradation system which prevents accumulation of damaged proteins like oxidizing proteins, aggresome-hydrophobic aggregates of damaged proteins that cannot be degraded by proteasome- and lipofuscin- known as the age pigment. [9] The decrease in the efficiency of this system and proteasomal activity with aging leads to formation of protein aggregates (aggresome) found in age-related neurodegenerative diseases. [9] Nevertheless, there is no clear evidence if the protein aggregation induces aging or if It is the other way around. [9]

Another popular damage theory is genome instability and it results from the failure of DNA repair mechanisms. [9] The stability and integrity of DNA are affected by DNA replication errors and physical, chemical and biological agents for which a complex repairing system exists with DNA helicases having a main role in nuclear repair events such as transcription

coupled repair, nucleotide excision repair and telomere maintenance. [9] Telomeres are repeated DNA sequences at the ends of linear chromosomes which are not fully replicated by DNA polymerases. [9] As a consequence, in each cell division there is a telomere shortening which can be reverted by telomerase action, if existing. [9] Otherwise, the cell will reach its maximum proliferative capacity due to telomere exhaustion. [9] The existence of a multiprotein complex named shelterin also prevents telomere repair by binding to telomeres and acting as a barrier to the action of DNA repair proteins. [9] Other contribution to aging (which still needs further studies for clarification) comes from mutations and deletions in mtDNA which is ten times higher than in nuclear DNA and enhanced by oxidative microenvironment and the absence of protective histones, which participates in damage repair in nuclear DNA. [9]

Other damage theory to be considered and also needing a better understanding it is the formation and the mechanisms behind the impact in mitochondrial and cellular function of the reactive nitrogen species (RNS), like nitric oxide. [9] RNS are suggested to be implicated in age- related diseases such as hepatic steatosis and apoptosis, cardiovascular system changes, impairments in sleep homeostasis, psychological disorders and dementia. [9]

Linked to cellular oxidative damage are the advanced glycation end-products (AGE) which act both dependent and independent from its cellular receptor (RAGE) and originating pro-oxidative, inflammatory and chemical actions. [9] AGEs formation results from a chemical reaction between carbohydrates and proteins (Maillard reaction) and they are used in food industry to add flavor and color. [9] AGEs have been associated with some diseases like diabetes, cardiovascular diseases and cognitive impairment but it needs to be better explored its role in the aging process. [9]

There has been an effort to develop an integrating theory to explain the causes of aging with several proposals without consensus. [9] The different suggested mechanisms seem to interact with each other in some point. [9]

What seems to be clear is the existence of repair/removal biomechanisms (by enzymes) to side products from unwanted chemical reactions during normal metabolism. [9] The level of accumulation of the remaining modified biomolecules/ alterations after repair defines the age-related changes extension and the expression of some with aging and corresponding interactions are already described. [9]

1.3. Hallmarks of aging

Aging occurs physiologically through a slow process of balanced functional decline across various organs in a coordinated manner (healthy aging) leading to longevity or through

tissue-specific premature aging resulting in chronic diseases and early deaths with short lifespans. [11] Centenarians manifest healthy physiological aging without outstanding tissue-specific aging and diseases which is not the case of the majority of the population, who suffers tissue-specific premature aging and related diseases. [11]

The aging process has interacting mechanisms (hallmarks) already investigated in human studies and which can be modifiable [4]. These hallmarks are identified through three main criteria, such as manifestation during normal aging, its experimental worsening should accelerate aging and its experimental amelioration should retard normal aging, increasing healthspan (this last criterion is the most difficult to achieve). [12] The extensive interconnection between the aging hallmarks imply that experimental amelioration of one will have impact on others. [12]

There has been made a categorization of some main hallmarks into:

- Primary: act as instigators of cellular damage, including genomic instability, telomere attrition, epigenetic alterations and loss of proteostasis. [1]

- Antagonistic: act opposing the damage done to the organism but can contribute to it when exacerbated or persistent, including deregulated nutrient sensing, mitochondrial dysfunction and cellular senescence. [1]

- Integrative: emerge when both primary and antagonistic damage cannot anymore be reversed by the organism homeostatic mechanisms, being responsible for the physiological phenotype observed with age. [1] This category includes stem cell exhaustion and altered cellular communication. [1]

1.3.1. Genomic instability

Genomic instability results from accumulation of genetic damage affects DNA integrity and stability. [4] [12]

This damage is caused by exogenous physical, chemical and biological agents and endogenous events like DNA replication errors, spontaneous hydrolytic reactions and ROS. [12] The damage can be point mutations, translocations, chromosomal gains and losses, telomere shortening and gene disruption by integration of viruses or transposons. [12] There has been an evolution of a complex network of DNA repair mechanisms to deal with these events but its failure causes the referred damage accumulation and related effects. [12]

Genomic instability can happen at:

- Nuclear DNA level: originates defects in transcriptional pathways that result in dysfunctional cells, being in the origin of Werner syndrome, Bloom syndrome, xeroderma pigmentosum, trichothiodystrophy, Cockayne syndrome, Seckel syndrome; [12]

- mtDNA level: usually thought mutations are induced by the oxidative microenvironment, lack of protective histones and lack of efficiency in repair mechanisms, it was found that most

mutations in adult cells come from replication errors in early age rather than from oxidative damage; [12]

- Nuclear architecture level: damage in nuclear lamina cause aging syndromes like Hutchinson- Gilford and Néstor- Guillermo, lamin A and telomere maintenance are interconnected and decrease in lamin B1 level is observed during cell senescence; [12]

Genomic instability is responsive to dietary energy restriction and increased exercise; [4]

1.3.2. Telomere attrition

Telomeres are repetitive nucleotide-sequences that protect chromosome ends from deterioration or fusion with adjacent chromosomes. [10] Telomere attrition is the shortening of telomeric DNA and the DNA damage to telomeres [4]. Shorten of telomeres results from the loss of around 50-200 pair of bases of unreplicated DNA at the 3' end in each cell division at a higher rate than the capacity of enzyme telomerase (terminal transferase) to add bases to the end of telomeres, failing to compensate their erosion. [10] DNA damage to telomeres is mediated by oxidative stress. [10]

Telomere attrition is one of the most important causes for induction of cellular senescence. [10] Short and dysfunctional telomeres resulting from repeated DNA replication in the absence of telomerase, triggers a persistent DNA-damage response that induces cell cycle arrest and the expression of senescence-associated secretory phenotype (SASP) [4] (see cellular senescence for details in SASP). Most of mammalian somatic cells do not express telomerase which explains the progressive and cumulative loss of telomere-protective sequences from chromosome ends, observed during normal human aging. [12]

Telomeres are bound by a multiprotein complex named shelterin which prevents DNA repair proteins to reach them and make chromosome fusions. [12]

Telomerase deficiency is associated with diseases like pulmonary fibrosis, dyskeratosis congenita and aplastic anemia due to loss of tissue regenerative capacity, while shelterin mutations cause telomere uncapping and chromosome fusion, being present in diseases like aplastic anemia and dyskeratosis congenita. [12]

Telomere attrition is responsive to Mediterranean, plant-based diet and antioxidant supplementation. [4]

1.3.3. Epigenetic alterations

Epigenetic alterations are changes in DNA methylation, noncoding RNA, histone modification and transcription, [4] chromatin remodeling. [12]

There is no direct experimental demonstration of impact in lifespan extension by DNA methylation, existing both descriptions of hypo-and hypermethylation of DNA with age. [12]

During aging occurs increase transcriptional noise and aberrant production and maturation of many mRNAs with impact in inflammatory, mitochondrial and lysosomal

degradation pathways. [12] This affects also noncoding RNAs, like microRNA (miRNA), known to influence the aging process and lifespan. [12]

The influence on aging by the manipulation of histone-modifying enzymes needs clarification whether it is by impact on DNA repair or transcriptional alterations affecting metabolic or signaling pathways outside the nucleus. [12]

There is an interconnection between DNA methylation, histone-modifying enzymes and heterochromatin protein and chromatin remodeling factors, impacting changes like global heterochromatin loss and redistribution which are characteristic features of aging. [12]

Reversion of epigenetic changes is possible and might have neuroprotective effects and induce epigenetic memory of longevity. [12] Epigenetic alterations are responsive to supplementation with folate and polyphenol and dietary energy restriction. [4]

1.3.4. Loss of proteostasis

Protein homeostasis (proteostasis) consists in mechanisms for the stabilization of correctly folded proteins and mechanisms for degradation of proteins by ubiquitin-proteasome system and autophagy-lysosomal system with additional regulators of age-related proteotoxicity acting in alternative pathways, all of them contributing to prevent the accumulation of damaged components. [12]

Proteostasis is altered during aging [5] [12] and proteome stability is associated with naturally long lifespan (example, in the naked mole-rat). [5] The loss of proteostasis affects protein folding, degradation and repair by ubiquitin proteasome [4], lysosome autophagy [4] and the IIS signaling pathways [5] affect the synthesis of chaperones. [4] [5] Age-related failure in proteostasis originates neurotoxic peptides associated with neurodegenerative diseases [5] [12] and also cataracts [12].

Loss of proteostasis is responsive to dietary energy restriction and intermittent fasting. [4]

1.3.5. Deregulated nutrient sensing

Nutrient sensing systems detect intra and extra cellular nutrients, such as by insulin/insulin like growth factor (IGF-1) intracellular signaling pathway (IIS/ILS), mammalian target of Rapamycin (mTOR) signaling pathway, sirtuin [4] [5] [12] and AMP-activated protein kinase (AMPK) [12].

IIS pathway participates in glucose sensing, it is the most conserved aging- controlling pathway in evolution and its most relevant targets related with longevity are the transcription factors FOXO family and mTOR complexes. [12] Multiple genetic manipulation in animal models that attenuate signaling intensity at different levels of the IIS pathway result in extension of lifespan, with mediation of the beneficial effects of dietary restriction on longevity. [12] Organisms with constitutively decreased IIS can survive longer due to lower rates of cell growth and metabolism which cause less cellular damage. [12] Also, as a defensive response to

physiologic or pathologic aging, it can be observed a decrease in IIS although extremely low levels of IIS signaling being incompatible with life. [12]

mTOR participates in high amino acids concentrations sensing and regulate all aspects of anabolic metabolism. [12] Leucine, arginine and methionine increased levels are activators of mTOR and also the cholesterol (through lysosome mediated process). [11] The protein TOR integrates signals from growth factors, nutrient availability, energy status and other stressors with effects in mRNA translation, autophagy, transcriptional and mitochondrial function for which there is evidence on the lifespan extension mediation. [5] It is thought that mTOR acts as a thermostat switching between physiological and pathological aging. [11] Genetic manipulation in animal models with downregulation of mTOR shows extension of longevity whilst the mTOR increase activity during animal models aging originates age-related obesity. [12] The inhibition of TOR activity (example, using sirolimus) during age has both beneficial and undesirable effects, because it can cause impaired wound healing, insulin resistance, cataracts and testicular degeneration (study in animal models). [12]

There is some genetic support to transpose animal model studies results to human concerning the findings that the inhibition of components of the ILS/mTOR pathways extends lifespan. [5]

IIS/mTOR sense nutrient abundance and anabolism and, in opposition, sirtuins and AMPK sense nutrient scarcity and catabolism. [12]

Sirtuins sense low energy states by detecting high NAD+ levels. [12] Sirtuins act by removing acyl groups from lysine residues on target proteins, having an important role in metabolism regulation that controls the response to calorie restriction and protecting against age-associated diseases, increasing healthspan and lifespan; [5] The beneficial effects of sirtuin are complex and interconnected, and include improving genetic stability and enhanced metabolic efficiency. [12] There is evidence that the activity of sirtuin decrease with age, during senescence and in animals with high fat diet. [5] Upregulation of sirtuins and AMPK favors healthy aging. [12] AMPK activation affects metabolism and shuts off mTOR, with positive response in the use of metformin for extension of lifespan in animal models. [12]

The deregulated nutrient sensing is responsive to low protein intake and dietary restriction. [4] [5]

1.3.6. Mitochondrial dysfunction

Mitochondrial dysfunction can accelerate aging in mammals although is not clear if the mitochondrial function improvement can extend their lifespan. [12]

The decreased number of mitochondria originates a mitochondrial disfunction in energy demand, accumulation of reactive oxygen, lipid peroxidation, impaired mitophagy. [4] Telomeres and sirtuins have a control role in mitochondrial function (protecting against age-

related diseases) acting on both biogenesis and clearance of damage mitochondria by autophagia. [12] The accumulation of mutations and deletion in mtDNA, oxidation of mitochondrial proteins, changes in membranes, among others changes, also cause defective bioenergetics. [12]

Although high levels of free radicals are implicated in cellular damage and inflammation, when present at lower levels they can increase cellular defenses through an adaptive response (mitohormesis) that is sensitive to mitochondrial perturbation triggering a nuclear transcriptional response related to protein folding, antioxidant defenses and metabolism; [5] the studies on the impact of mitohormesis in extending lifespan challenge the use of anti-oxidants as a strategy for lifespan extension. [5]

Mitochondrial dysfunction is responsive to dietary restriction and aerobic exercise; [4] [12]

1.3.7. Cellular senescence

Cellular senescence is a permanent state of cell cycle arrest that occurs in diploid cells (proliferating cells), which fail to re-enter the cell cycle in response to mitogenic stimuli. [10] The senescent cells remain metabolically active, although with a changed cell metabolism, presenting glycogen accumulation, increased glucose consumption and increased lactase production. [10]

The main features that characterize senescent cells are arrested cell proliferation, resistance to apoptosis and secretion of SASP. [5] SASP composition includes: immune modulators; inflammatory cytokines (interleukin(IL)-6, IL-8 and IL-1α); growth factors (insulin-like growth factor-binding proteins (IGFBP); chemokines (CXCL-2, CXCL-3 and CXCL-5, among others, present in oncogene induced senescence (OIS)); proteases (urokinase or tissue type plasminogen activators, etc.); matrix metalloproteinases (MMP-1, MMP-3); NO; ROS. [10]

Additionally, senescent cells present altered cell size (more smoothed shape), senescence-associated heterochromatin foci formation, lipofuscin accumulation, DNA damage foci, loss of lamin B1, senescence-associated distension of satellites, expression of embryonic chondrocyte-expressed 1 (DEC1), expression of decoy death receptor 2 (DCR2), upregulation of some miRNAs, higher activity of senescence- associated β-galactosidase (SA-β-gal). [10]

Causes of cellular senescence are short and dysfunctional telomeres, oncogene activity (OIS by replication stress and DNA damage), epigenomic perturbations and mitochondrial dysfunction. [5] Cellular senescence can also be triggered by augmented levels of p16INK4a, p21CIP1 and p27 (which act as cell cycle inhibitors), higher expression of p19ARF, p53 and PAI-1, augmented NF-κB signaling. [10] All these features define the gold-standard markers to identify senescent cells. [10] Stress- induced senescence occurs through oxidative stress, endoplasmatic reticulum stress or interferon (IFN)- related responses. [10] Some therapy-

induced senescence occurs with UV, γ-irradiation, tert-butyl hydroperoxide (DNA damage agents) or anticancer chemotherapy agents. [10]

The primary purpose of senescence is the prevention of damaged cells propagation and trigger their death by the immune system [12], representing a physiologic response to prevent genomic instability and accumulation of DNA damage [10]. This process requires an efficient cell replacement system (clearance of senescence cells and cellular regeneration) otherwise the senescent cells will accumulate causing damage and aging. [12] The secretion of SASP has a negative impact in tissues and affects clearance by inflammasome. [4] Through SASP, senescent cells influence the tissue microenvironment with effects in neighboring proliferating cells and recruitment / activation of immune cells in aging tissues and tumors. [10]

Senescent cells are more abundant in aged and diseased tissues with a variety of studies (cell culture and animal models) demonstrating its relation and SASP with large number of age- related pathologies, like neurodegenerative diseases, atherosclerosis, cardiovascular dysfunctions, tumor progression, loss of stem cell function, non-alcoholic fatty liver disease, pulmonary fibrosis, osteoarthritis, osteoporosis. [5] Nevertheless, studies in animal models show that cellular senescence is not a generalized property of all tissues in aged organisms, being observed in liver, skin, lungs and spleen but not in heart, skeletal muscle and kidney. [12]

Despite this involvement in pathological conditions and being one of major causes of aging, senescent cells also have an important and positive role in physiological processes such has embryogenesis, tissue remodeling and tissue repair, working also as a potent barrier to prevent tumorigenesis. [10] This might be an explanation for natural selection (which provides for an optimal survival and reproductive success of the organism) to allow cellular senescence to be transmitted to offspring, going in favor of antagonist pleiotropy hypothesis [10], presented in the theories of aging chapter.

Cellular senescence is responsive to senotherapy (clearance of senescent cells) and prevention of senescent cells accumulation using metformin in human cells models. [4]

1.3.8. Stem cell exhaustion

Stem cell exhaustion consists in the decrease of the regenerative potential of stem cells. [4] Animal model studies show that functional attrition of stem cells happens is all adult stem cell compartments, including forebrain, bone, muscle fibers, hematopoietic. [12] This can be caused by accumulation of DNA damage, overexpression of cell-cycle inhibitors proteins ($p16^{INK4a}$), telomeres shortening, among others. [12] On the other hand, the excessive proliferation of stem cells and progenitor cells can cause the exhaustion of stem cell niches and premature aging which can be influenced by fibroblast growth factor 2 (FGF2) signaling. [12]

Stem cell exhaustion is responsive to regenerative medicine based on mesenchymal stem cells, musculoskeletal damage repair. [4]

1.3.9. Altered intercellular communication

Changes at the level of intercellular communication are related to deregulated endocrine, neuroendocrine neuronal signaling associated with chronic inflammation, decline of adaptive immune system and others. [4]

During aging, increased inflammatory reactions, decreased immunosurveillance against pathogens and premalignant cells and changes in the composition of extracellular environment originate deregulation of neurohormonal signaling such as renin-angiotensin, adrenergic and insulin IGF-1. [12]

Inflammaging is a low level and permanent state of inflammation resulting from the accumulation of proinflammatory tissue damage, the dysfunctional activity of immune system clearing pathogens and dysfunctional host cells, the senescent cells secretion of proinflammatory cytokines, increased activation of nuclear factor- kappa B (NF-κB) transcription factor and defective autophagy response. [12] This scenario induces increased production of IL-1β, tumor necrosis factor and interferons and originates pathogenesis in obesity, type 2 diabetes, atherosclerosis, inhibition of epidermal stem cell function. [12]

The declining of adaptive immune system (immunosenescence) has serious impact in accelerating aging phenotype at systemic level and it is characterized by failure in clearing infectious agents, infected cells, cells in premalignant state, senescent cells, hyperploid cells and premalignant lesions. [12]

Hypothalamus regulates other relevant inflammatory pathway for aging through the overactivation of NF-κB pathway, which can be modulated by genetic or pharmacologic therapeutic. [12] The induced signaling of NF-κB pathway will impact gonadotropin-releasing hormone (GnRH), reducing its secretion with consequences to age-related changes like bone fragility, muscle weakness, skin atrophy, reduced neurogenesis. [12]

Adenylate-uridylate-rich element RNA-binding Protein 1 (AUF1) is a mRNA decay factor implicated in the cessation of inflammatory response which also activates telomerase subunit TERT, contributing for telomeres maintenance with reduction of aging process. [12]

Sirtuins also slows down aging through deacetylation of histones and NF-κB resulting in a downregulated inflammatory response. [12]

There is evidence that age-related changes in one tissue induces ageing-specific deterioration of other tissues, that senescent cells induce senescence in neighboring cells and CD4 T cells acquire functional defects through microenvironment. [12]

The altered intercellular communication is responsive to gastric bypass, caloric restriction and resistance exercise. [4] Other interventions to be considered might be use of blood- born systemic factors, long term administration of anti-inflammatory agent (example, acetylsalicylic acid), manipulation of gut microbiome (effects in immune system). [12]

1.3.10. Chronic inflammation (inflammaging)

Older organisms tend to have higher levels of inflammatory markers in cells and tissues resulting in a low-grade, sterile and chronic pro-inflammatory status linked to several age-related diseases, like cancer, type 2 diabetes, cardiovascular diseases, neurodegenerative diseases and frailty. [5] [1] Causes of inflammaging are immunosenescence (senescence of immune system), genetic susceptibility, obesity, oxidative stress, changes in the intestinal barrier permeability, chronic infection, defective immune cells, SASP from non-immune senescent cells, environmental chemicals. [5] [1]

Inflammaging is responsive to dietary restriction. [5]

1.3.11. Loss of circadian behavioral patterns (sleep-wake cycles)

During aging, there is reduction of the circadian gene expression; [5] the loss of circadian behavioral patterns has impact on NAD+ levels and sirtuin activity as well as other biological processes modulated by circadian clocks being linked to age-related pathologies like neurodegeneration, obesity and type 2 diabetes. [5]

1.4. Genetics

Human population is characterized by a large genetic heterogeneity that has a critical role in disease susceptibility, lifespan and response to therapy by an individual. [5] This originated the new field precision medicine which aims to identify disease critical genetic determinants and to customize interventions and treatments to unique genetic variants. [5] Precision medicine and geroscience need to interact closely. [5]

From multiple genome-wide association studies (GWAS) performed on longevity, the only genetic locus showing a genome-wide significance is apolipoprotein E (APOE). [4] The APOE is a cholesterol carrier in peripheral tissues and brain which is associated with cardiovascular and Alzheimer's diseases. [4] [5] Families show 1-10% longer lifespan in their generation, lower risk of some diseases (coronary artery disease, cancer and type 2 diabetes) and better immunity and metabolism when compared with general population. [4] Nevertheless, it cannot be excluded the influence of common, non-genetic influences in early life of these families. [4] From GWAS there is also reference to *INK4a/ARF* locus as the locus genetically linked with the highest number of age-related pathologies such as cardiovascular diseases, diabetes, glaucoma and Alzheimer's disease. [12] The *INK4a/ARF* locus codes for p16/p19- p53 proteins expression. P16 protein levels correlate with chronological age for a generality of tissues analyzed. [12] Both p16/p19 and p53 act as an inducing mechanism for cellular senescence responding to oncogenic insults to avoid the propagation of damaged cells. [12] [10] This action needs to couple with tissue regenerative capacity to have benefits and when it gets exhausted the *INK4a/ARF* response become deleterious and accelerate aging. [12]

Functional genetic variants of the IGF-1 receptor were identified in individuals from long-lived families. [4] [5]

Informative studies in model organisms have determined that *FOXO3A* locus has a consistent role in healthy aging. [4] [5]

Another gene known to be associated with human longevity is *SIRT6*. [5]

1.5. Interventions on aging process

Taking into consideration the multifactorial nature of aging process there are several different interventions under study to extend the healthy life and longevity. [9] Most of these interventions were observed in simpler organisms and need to be demonstrated as viable anti-aging therapies in humans. [9] [5]

Testing these interventions for human use can take advantage of geroscience concept in which the aging therapies will ameliorate or prevent several age- related diseases at the same time. [5] The clinical trials should use clinical outcomes like multimorbidity, combination of several age-related chronic diseases, geriatric syndromes like frailty or delirium, resilience to health stressors (surgery or infection), grip strength, gait speed, timed-up-and-go and daily living activities. [5] The timing for starting the aging process intervention in order to assure efficiency is another variable to study. [5]

1.5.1. Nutrition

In caloric restriction (CR) the food intake in laboratory animals is experimentally reduced, while avoiding malnutrition, being specific in the dietary components that are reduced, like some proteins, and given under a regime of intermittent fasting (example, meal given once a day) [2]. The extension of lifespan has been observed in these laboratory animals and several causes have been raised, like, increase of the antioxidant defense, optimization of metabolism due to limited calories available, decelerate of genetic program, hormonal and proteome changes [9]. It is thought that through the ILS/TOR pathway (nutrient sensors), there is a metabolic switch from reproduction and growth to somatic maintenance leading to lifespan extension. [5] Dietary restriction studies are starting to show it as a promotor of circadian homeostasis by increasing expression of rhythmic genes. [5] Nevertheless, several animal studies have challenged the universality of the benefits of dietary restrictions. [5] Studies comparing different diets and populations geographically associated with increased longevity (‘blue zones’) indicate that minimally processed foods, predominantly plant- based, low alcohol consumption and a lack of overeating are beneficial for longevity and healthspan. [5] New achievements are the intermittent fasting and ketogenic diet which also need more studies. [5]

1.5.2. Stem cell therapies

Although already in use for diseases treatment, it still needs a better understanding the stem cells mechanisms that influence aging process and there are technologic challenges for obtaining the right therapeutic. [9]

1.5.3. Breaking AGEs

Additional to dietary AGEs restriction and exercise, there are already some pharmacologic studies to block the reactions that form AGEs or their actions using, among others, aspirin and metformin (see below) but the side effects need further studies before a safe use can be done. [9]

1.5.4. Hormonal therapies

Human growth hormone (GH) has started to be used as an anti-aging agent but its adverse side effects put it on hold until more research is done on a safe use. [9]

1.5.5. Antioxidants

Use of antioxidants (vitamins A, C and E and co-enzyme Q10) in dietary supplements and creams, although widespread, has not be proven to have beneficial effects on delaying aging [9].

1.5.6. Telomere- based therapies

Telomere measurement kits are being used to estimate biological age but there is little evidence to support this claim. [9] Some therapy to activate telomerase has shown some health benefits but increased risk of cancer development needs to be considered. [9]

1.5.7. Other pharmacologic therapies

- Metformin: this is a widely used antidiabetic drug that also targets several aging molecular mechanisms; [5] there is evidence of increase in lifespan, prevention of diabetes onset, improvement of cardiovascular risk factors, reduced mortality and it is suggested that metformin might reduce cancer incidence and neurodegenerative diseases; [5] it is being organized the clinical trial Targeting Aging with Metformin (TAME), targeting 65- 80 years old population without diabetes but at high risk of age-related chronic diseases development. [5]
- Sirolimus (immunosuppressant) inhibits the mTOR pathway and insulin/insulin-like growth factor signaling (IIS) acting on the management of energy and nutrients and, in animal models, has shown results in extending maximum lifespan. [2] [9] [5] Nevertheless, there are serious adverse effects (example, nephrotoxicity) and more investigation is being done on the use of mTOR pathway to influence ageing. [9] Two six weeks clinical trials targeting immunosenescence using non-immunosuppressive dose of rapamycin and the analogous

drug everolimus showed improvement in immunological response to vaccines with reduction of infection rates during a period. [5]

- Senolytics: these are drugs that selectively target and eliminate senescent cells with great geroprotective potential in animal models which are starting to be tested for safety in humans with no results, until now, on efficacy. [5]
- Sirtuin activators: sirtuin activating compounds (STACs) Resveratrol (natural STAC) and SRT1720 (early synthetic STAC) have failed clinical trial (low bioavailability, potency and limited target specificity) but the new molecule SRT2104 shows high specificity to SIRT1 and has been used in small clinical trials related to cardiovascular and metabolic markers, type 2 diabetes, cigarette smokers. [5] Larger clinical trials are being prepared. [5]
- Genetic therapy by modulating aging- related genes (like the gene *klotho* whose overexpression in mice shown extension of lifespan, and for which resveratrol has been described as an inducer) is another field of research in the extension of lifespan. [9]
- Precursors of nicotinamide adenine dinucleotide (NAD+): it is under animal models research the use of supplementation with NAD+. [9] NAD+ acts as co-substrate for sirtuins and its levels decrease with age, senescence and in animals with high- fat diet. [5] NAD+ levels increase in conditions that increase both healthspan and lifespan (like dietary restriction and exercise) and decrease during aging or conditions that decrease healthspan and lifespan (like high fat diet) which support the idea of NAD+ levels being part of the aging process and the use of NAD+ supplementation as protective measure during aging, despite the lack of demonstration until now of its efficacy in humans. [5]

1.5.8. Exercise

Exercise has demonstrated an effective geroprotection, reducing the incidence of age-related diseases, improving life quality and increasing mean and maximum lifespan in humans, having positive impact even with modest implementation. [5] The molecular pathways by which exercise benefits are obtained are still unknown. [5]

1.6. Aging and age-related diseases biomarkers

The rate of aging can be variable and is influenced by genetic, lifestyle, environmental and interaction factors. [13]

A set of biomarkers have been defined to detect individual variability to the progress of aging (indicators to determine an individual's health status and aging disease risk) and to monitor the response to interventions, allowing also to define the biological age (although no consensus still exist for these biomarkers), that is the stage of aging process despite the chronological age. [4] [13] Some examples are biomarkers for physical and mental capability (locomotor function, strength, balance, cognition, etc.), respiratory and cardiovascular function, ability to regulate lipid and glucose metabolism, immunity. [4]

Under evaluation (comparison between each other and with traditional parameters) are biological age biomarkers based on age-related changes at cellular level- transcriptome, epigenome, metabolome, structural neuroimaging- such as telomere shortening, epigenetic clocks [4] [5], inflammaging [5]. Additionally, the accumulation of senescence cells in tissues can be inferred by surrogate markers such as DNA damage and SA-β-gal [12] or C-reactive protein (CRP) and IL-6 for inflammaging state detection [1].

1.6.1. DNA methylation

Although telomere length in blood has been used as a biomarker, also associated with diseases, risk factors and mortality, some conflicting results have been reported and epigenetic clocks, which measures DNA methylation patterns status, have been put forward as a more accurate predictor. [13]

DNA methylation affects the phenotype without changing the genotype through the methylation of cytosine nucleotide if coupled with guanine nucleotide (named CpG site). [13] These CpG dinucleotides are unevenly distributed across the genome, either isolated or clustered (named CpG islands- CGIs). [13] The function of methylation is dependent of the context and genome location, either repressing transcription or activating genes and recruiting transcription factors. [13]

Despite DNA methylation patterns being maintained by DNA methyltransferases, changes accumulated with age are observed, in which older individuals present more variable DNA methylation patterns compared to younger individuals. [13] Also, aging is accompanied with a progressive loss of DNA methylation in CpG poor regions [13] [14], leading to global hypomethylation, activation of proto-oncogenes and chromosomal instability [14]. At the same time, there is an aging correlated hypermethylation of CGIs. Age associated methylation changes occur both randomly in the genome (named epigenetic drift) and age-specific methylation changes which occur at specific genomic locations, often shared across different tissue and cells. [13]

Using methylation status of these multiple age-associated CpG sites, different models of epigenetic clocks were developed, such as Hannum's (using whole blood samples), Horvath's (using multiple tissue samples) and Weidner's (focus in three specific CpG sites) to use this measure of DNA methylation age acceleration as indicator of biological age. [13] The DNA methylation age measures the cumulative degradation of the epigenetic maintenance system and it is not a marker of cellular senescence because DNA methylation age advances also in non-proliferative or immortal cells. [14]

The accurately and reliable quantification of aging-associated epigenetic changes will allow to study the etiology of several aging diseases (example, cancer or neurodegenerative diseases) and drug targets in biomedical research, evaluate the efficacy of antiaging

interventions (example, caloric restriction), evaluate risk of all-cause mortality, enhance forensic and archeological analysis. [14]

1.6.2. miRNAs

Circulatory miRNAs are another promising biomarker for aging and aging-related diseases, having a role in the diagnosis, pathogenesis and therapeutics. [15]

Their potentiality uses for aging and aging -related diseases assessment comes from their stability and abundance in the extracellular circulation and the fact of being found in various diseases as cancer, viral infections, diabetes, immune-related diseases, cardio-cerebrovascular disease, neurodegenerative disorders, etc. [15]

miRNAs production starts in the cell nucleus and matures at cell cytoplasm, afterwards they work as gene modulator or are released in the extracellular biofluids, such as blood, serum, plasma, saliva, urine, among others. [15]

miRNAs circulate in extracellular spaces in five different ways, such as, bound with high-density lipoprotein particle (minor fraction of transportation), in a complex form with Ago2 protein (around 90% of circulatory miRNAs), packaged within exosomes (nanovesicles originated from endosomes and secreted from plasma membranes), encapsulated within micro-vesicles (larger vesicles generated directly from the plasma membrane of different cell types- neurons, muscle cells, inflammatory cells, tumor cells and, their major source, platelets) and accumulated in apoptotic bodies (larger vesicles released from cells during apoptosis). [15]

The assessment is made through the quantification of circulatory levels of aging specific miRNAs (comparison of the elderly with younger individuals) and disease specific miRNAs/ expression deregulation. [15]

Some advantages of miRNAs use as biomarker are the non-invasive data collection, their stability, measurable analytes and specificity for disease, tissue and pathway. [15] Some challenges still to be overcome are the interindividual variation, lack of standardization and clinical validation, need for standardization of reference level and therapeutic limit. [15] More population-based studies are needed for the analytical standardization of miRNAs and improvement of their clinical utility in the management of aging diseases. [15]

1.7. Age- related physiologic changes and diseases

Aging is currently defined as a progressive loss in tissue and organs functions over time. [10] The aging physiologic changes start at sub-clinical level from the thirties on, affecting the body composition (loss of bone, cartilage, muscle mass, strength and gain of abdominal fat), hormonal changes, blood pressure, blood lipids, vascular stiffness [4], stride intervals, respiratory cycles, vision, postural dynamics, decreased fertility, among others [9]. These sub-clinical changes might originate diseases from middle age on, causing multimorbidity and frailty. [4]

1.7.1. Cardiovascular system

The heart cells population (cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, endothelial cells, cardiac stem cells) suffers a decline with aging leading to increased fibrosis which is associated with left ventricular hypertrophy and stiffness, which is a prominent feature of heart failure. [16] The ventricular diastolic function declines with advancing age but not the systolic function. [16] At the same time, there is a decrease in ventricular cavity size. [16] The aging correlation with this remodeling is also affected by additional risk factors such as higher body mass index, blood pressure and diabetes. [16] As for the atrial remodeling, there is an increased diameter (volume) and myocardial fibrosis. [16] The atrial reservoir and conduit functions decline with age. [16] The pericardium also suffers an increased fibrosis and deposition of adipose tissue. [16] The autonomic nervous system which regulates cardiovascular function originates a decrease in the intrinsic heart rate. [16]

There is an endothelial disfunction at the level of medium-sized and small vessels and central arterial stiffness (at large artery level) due to reduction of NO vasodilator effect and calcification of the vessels. [16] It is observed a remodeling of aortic root with wider aortic diameter and longer length of the ascending thoracic aorta associated with increased risk of heart failure. [16] Pulmonary circulation changes with age is less known than systemic circulation but there is evidence in increased pulmonary artery systolic pressure (with implications in morbidity and mortality) and pulmonary vasculature seems to suffer similar remodeling as the systemic circulation. [16]

In the elderly, aortic stenosis is the most prevalent valvular disease in developed countries, caused by degenerative valvular heart disease which progressively originates calcification, narrowing and stiffness of the valve. [16] Other valvular heart diseases age-related are aortic valve sclerosis (due to endothelial dysfunction), mitral stenosis (due to calcification of mitral annular) and mitral regurgitation (due to imperfect mitral leaflet closure). [16]

Atherosclerosis results from plaques accumulation in the arteries (composed of lipid-containing, foamy macrophages), which is induced by an inflammatory response that recruits monocytes when lipoproteins accumulate in the intima of arteries activating endothelial and vascular smooth muscle cells. [10] When these plaques are eventually released into the bloodstream it will cause thrombosis and damages in the organs fed by the circulatory system, such as heart, brain and kidney. [10] Activated endothelial and vascular smooth cells upregulate some cellular senescence biomarkers (SA-β-gal, p16, p21) originating contradictory results because while the senescence stops the monocytes and macrophages plaques growth, the secretion of SASP by those senescent cells induces the disease progression. [10]

1.7.2. Kidney

There are significant changes in structure and function of the healthy aging kidney. [17] The cortical volume decreases but there is an increase in medullary volume until age of 50, after which there is a decline in global kidney volume of 22cm^3 per decade. [17] Other changes are the increase in the surface roughness, in the sinus fat and in the number and size of simple renal cysts. [17] These findings can be attributed to underlying nephrosclerosis with nephron loss, interstitial fibrosis, glomerulosclerosis and hypertrophy of remaining tubules. [17] With the decline of nephron number there is a comparable reduction in measured whole-kidney glomerular filtration rate (GFR) but not of the single nephron GFR, which remains relatively constant with healthy aging as does glomerular volume. [17] The single-nephron GFR increases only when the glomerulosclerosis and arteriosclerosis exceed what is expected for the age. [17]

Most of chronic kidney disease found in the elderly is due to fit currently criterium of estimated GFR<60ml/min per 1.73 cm^3 of body surface area (and not by criterium urine albumin: creatinine>30mg/g) which matches the average measured GFR in a representative cohort of community living adults aged over 70. [17] This leaves a substantial number of elderly likely to have only physiologic aging of kidneys. [17] Nevertheless, the lowest risk of mortality for chronic kidney disease with absence of abnormal albuminuria for ages >75 years old is with a GFR of 45–104 ml/min per 1.73 m^2 of body surface area. [17]

1.7.3. Liver

Age-related changes in liver function have an impact in systemic susceptibility to age-related diseases because of liver regulation of systemic energy metabolism (hepatic glucose and lipid homeostasis), steroid biosynthesis and degradation, and insulin signaling. [18] Due to these functions, there is an hepatic important role in nutritional interventions (caloric and protein restrictions) on age and age-related diseases but, if there is a dysregulation of hepatic energy metabolism, liver will participate in age-related conditions such as insulin resistance, diabetes mellitus and non-alcoholic fatty liver disease (NAFLD). [18]

All hallmarks of aging described in chapter 1.3 have a direct impact in all different hepatic cells. [18] The number of hepatocytes decreases (due to inhibition of progenitor liver cells by oxidative stress) with impact in liver regeneration and repair capacity and senescent hepatocytes have increased lipid droplet accumulation, decreased mitochondrial oxidizing capacity and increased production of ROS, contributing to increased incidence of NAFLD and non-alcoholic steatohepatitis in older people. [18] Also, decline of autophagy results in an inadequate removal of damaged proteins with an accumulation of lipofuscins being observed in aged hepatocytes. [18] Aged liver endothelial sinusoidal cells (LSECs) loses fenestrations (reduction in number and size) increasing the susceptibility to cardiometabolic diseases through impaired uptake of lipoproteins and insulin. LSECs endocytotic activity is reduced,

such as clearance of circulating collagen degradation products, hyaluronan and antibodies. [18] Impact of age in hepatic stellate cells is less studied existing uncertainty about their activation (associated with development of hepatic fibrosis and cirrhosis) in old age. [18] Küpffer cells are resident liver macrophages for which there is not much research on the effects of aging but some studies on rat model shows increased number, reduced phagocytotic activity, increased expression of inflammatory IL-6 and contribution to the pro-inflammatory state of the hepatic sinusoid. [18]

1.7.4. Lung

Aging originates a decrease in the functional capacity of the lungs. [19] The loss of elasticity cause enlargement of airways with increased resting functional residual capacity and increased end-expiratory lung volume. [19]

Lung diseases incidence as chronic obstructive pulmonary disease (COPD), pulmonary fibrosis and pneumonia increase with age. [19] COPD result from age-related changes in lung capacity, being accelerated by cellular stressors such as pollution, particulates, cigarette-smoke (inducer of senescence). [19] As soon as COPD occurs, all hallmarks of senescence appear. [19] Pulmonary fibrosis is associated with cellular senescence and mitochondrial dysfunction. [19]

The age-related cellular changes in lungs are: [19]

- Alveolar epithelial cells: contribute to susceptibility to lung infection and dysregulated repair responses;
- Fibroblasts: production of more collagen, fibronectin and matrix metalloproteinases, affecting elasticity and the airspace enlargement;
- Leukocytes: are present in the lungs and immunosenescence together with age-associated inflammation contributes to susceptibility and severity of influenza and pneumonia.

Having lung disease or pneumonia can accelerate the development of other diseases, such as type 2 diabetes and dementia. [19]

1.7.5. Type 2 Diabetes/ obesity

Expression of senescence in pancreatic β cells has been detected in animal studies by increased levels of biomarker p16 but a positive influence was observed in the function of these cells, increasing the production of insulin in the presence of glucose, indicating that pancreatic β cells senescence may not be a factor in age-associated type 2 diabetes. [10]

On the other hand, excessive caloric intake induces senescence in adipose tissue via ROS-mediated activation of p53 and p21, upregulating proinflammatory factors (CCL2 which is a macrophage recruiter resulting in infiltration of adipose tissue and secretion of more proinflammatory factors leading to insulin resistance and tumor necrosis factor-alpha (TNF-α) which interferes with glucose homeostasis) and downregulating anti-inflammatory factors

(example, adiponectin which induces glucose uptake and reduces gluconeogenesis with an overall reduction on glucose level) leading to impairments in insulin sensitivity and glucose tolerance in animal studies. [10] The same process with adipose senescence is suggested to occur with human insulin resistance as the same senescence markers are expressed in human adipocytes of diabetic patients. [10]

1.7.6. Bone

Osteoarthritis consists in a progressive degeneration of the articular cartilage originating bony projections (osteophytes), thickness of synovial ligaments and local inflammation causing chronic pain and movement difficulties. [10] The age-dependent senescence of chondrocytes is responsible for their articular cartilage maintenance capacity loss, with senescence markers like SA-β-gal, p16 and MMPs being detected. [10]

Osteoporosis consists in the loss of bone density and strength due to a bone resorption by osteoclasts higher than bone formation by osteoblasts. [10] Advanced age is one of the major risk factors for osteoporosis and senescent bone cells were defined as an inductor through the secretion of SASP which increases osteoclasts progenitor survival and impair bone synthesis. [10]

1.7.7. Neurodegenerative disorders

Neurodegenerative disorders like Alzheimer's and Parkinson's disease increase with age but although there are findings of a neuroinflammatory associated state with presence of some senescent cells biomarkers there is limited evidence in a causal link between senescence and neurodegeneration. [10] There is a common characteristic amongst their pathogeneses which is the failure to maintain intercellular protein homeostasis (proteostasis) occurring accumulation of aggregates. [20] Currently it is thought that elevated ROS/RNS in aging it is the primary causal factor added by the decrease in the efficacy of protein quality control mechanism that leads to impaired clearance of aggregate-prone proteins. [20] Additionally, it seems that each neurodegenerative disorder has a distinct aggregation proteomic profile. [20]

Glaucoma is characterized by an increased intraocular pressure, due to aqueous retention, causing retinal ganglion cell death with progressive degeneration of the optic nerve leading to blindness. [10] Advanced age is one of the leading risk factors and SASP secreted by senescent cells has a probable role in the microenvironment changes that limit the aqueous outflow. [10]

1.7.8. Immune system

Innate and adaptive immune systems are both affected by aging [21], being the adaptive system the more affected one [22]. This is known as immunosenescence and consists of several changes in the development and function of the innate and adaptive immune system

that leads to an increased susceptibility to disease in the elderly, although it can also occur age independently (example, by myeloid-derived suppressor cells in cancer) [10] and poorer response to vaccinations [22] . The principal characteristics of immunosenescence are persistent low-grade inflammation ('inflammaging'), decreased capacity for infection or cancer fighting, impaired ability to an effective response to new antigens, increased incidence of autoimmunity [1] [21] and impaired wound repair. [1] The study of immunosenescence should include, additionally to organism aging or cellular senescence, the cell ontogeny programs, cell-intrinsic defects, stromal microenvironment and the overall history of antigen exposure. [1]

The innate immune senescence occurs by reduction in antigen processing and presentation capacity, decreased response to stimuli and a chronic activation state. [10]

The adaptive immune senescence occurs by loss of T or B cell receptor repertoire diversity and impaired immunological memory formation. [10]

Age-related epigenetic changes in immune cells are likely to contribute to the development of autoimmune disease in those who are susceptible. [21]

The reduced capacity of innate immune system to present the antigen leads to a lack of stimulation of T-cell activation (both CD4+ and CD+8) with impact in B-cell proliferation and antibody production from adaptive immune system. [22]

Additionally, there is a defective function of lymphocytes with age which mechanisms have been extensively studied in mouse model, such as, among others, change in cell surface glycosylation, incapacity to adjust the threshold of response to IFN-1 receptor signaling and T-cell senescence with high secretory capacity. [1]

Immunosenescence development varies with age and existence of co-morbidities, becoming apparent after the 60-65 years and more significant after the 85 years of age. [22]

Innate immunosenescence

The change in innate immune system seems to be related with a basal activate state during aging, probably caused by a chronic low-level stimulation by infectious agents, characterized by increased pro-inflammatory mediators and decreased receptor signaling and effector function. [21]

Although neutrophiles have a short lifespan and die by apoptosis if unstimulated, in the elderly the pro-inflammatory stimuli can increase their lifespan and their number appears to be high but with altered effector functions. [21] Their ability to migrate to infection sites and phagocytosis activity are reduced. [22]

Monocytes and macrophages show decreased function such as cytotoxicity, intracellular killing, antigen presentation [21] and response to IFN-γ [22]. During aging, both monocytes and macrophages express on their surface lower levels of human leukocyte antigen (HLA) and major histocompatibility complex class II (MHC-II). [22]

Inflammatory monocytes subpopulation CD14+CD16+ is increased with higher pro-inflammatory cytokine production, such as IL-1β which is regulated by DNA-methylation. [21] Inflammatory genes in monocytes are regulated by DNA methylation, post-translational histone modifications, transcription factors of FOXP3, interferon regulatory factor (IRF), NF-κB and signal transducer and activator of transcription (STAT) families. [21]

The production of cytokines by macrophages is reduced, mostly IL-6 and TNF-α, due to change in expression of toll-like receptors (TLR) and, simultaneously, the development of defect in macro-autophagy leads to macrophage accumulation and corresponding inflammatory cytokines. [22]

Natural killer (NK) numbers increase with age although there is a reduction in cytotoxic activity against virus-infected and cancerous cells. [21] This is due to reduced expression of $CD56^{bright}$ receptor (immunoregulatory function) and increased expression of $CD56^{dim}$ receptor (cytotoxic action), resulting in a reduce responsiveness to cytokine signaling. [22]

Adaptive immunosenescence

Aging acts over the human hematopoietic system by decreasing the cellularity of the bone marrow, declining the adaptive immune response and increasing hematological disorders and malignancies. [1] This happens through changes in immune cell repertoire, cell intrinsic defects in lymphocytes and change of the immune environment from anti-inflammatory to pro-inflammatory in old age. [1]

Due to thymic involution and the decline of its function [1][22], such as defective production of cytokines and growth factors [22], adult human naïve T cell populations decrease [21] [22] and they are almost exclusively due to homeostatic proliferation [1]. This happens in an effective way for CD4+T cell pool during human healthy aging but with less efficiency for CD8+T cells, [1] and with a decrease in the T cell antigen repertoire (TCR) diversity which can also be induced by expansion of memory T cells [1] [21] [22] to respond to latent virus such as cytomegalovirus (CMV). [1] [22] The loss in TCR repertoire is responsible for the decreased response to vaccines, increased susceptibility to infections and reduced memory to previously encountered pathogens. [21] Thymus atrophy also decreases its capacity to establish a central tolerance, resulting in an increased number of self-reactive T cell circulation which activity aged regulatory T cells (Treg) cannot suppress. [22] Increase in the senescent T cells is caused by chronic antigenic stimulation [21] [22] from internal altered tissue and molecular debris or microorganismal material. [21]

B cells also undergo age-related reduction in their precursors in bone marrow [1], due to reduction of IL-7 production (acts as growth factor to B cells) [22], being observed a progressive shift from naïve B cells to homeostatic expansion of antigen- experienced cells in the peripheral B cell compartment, and a reduction in the repertoire diversity related with poor

general health. [1] Age-associated B cells (ABCs) have been described, which accumulate in the peripheral blood with age [1] [22] and do not proliferate after B cell receptor but undergo a robust proliferative response by receptors stimulation implicated in autoimmune responses, such as TLR-7 and TLR-9. [1] Aged B cells present defects in normal functions such as, ability to recognize and respond to new antigens, reduced diversity of B cell receptor and reduced capacity to differentiate into plasma cells with consequences to the capacity of antibody production. [22] At the same time, production of TNF-α occurs, contributing to the inflammaging. [22]

Immunosenescence and cancer

The advances of the immunotherapy use in cancer originated a new clinical discipline, immuno-oncology, having as therapeutic agents, antibodies, small molecules, adjuvants, cytokines, peptides, proteins, bi-specific molecules, oncolytic viruses, and cellular therapies. [1] It has been broadly successful in the treatment of various origins cancers and it is becoming an integrated part of the therapeutic approach to patients with cancer, introducing also new criteria to evaluate clinical response to therapy, called immune-related response criteria (irRC), and immune-related adverse effects (irAE)s. [1] The two major irAEs identified so far are immunotoxicity and autoimmunity. [1]

It is needed to understand the tumor-specific immunopathology in the context of immunosenescence as well as to define biomarkers to identify if adequate tumor-specific immunity response is obtained. [1]

The cancers majority emerge in elderly patients, however, this group is underrepresented in clinical trials and, when represented, good health status and adequate organ function participants are chosen. [1] Neither the data obtained from younger age groups can be automatically extrapolated to the elderly nor the results from good health older individuals apply to those with multiple co-morbidities or frailty. [1] It is imperative to develop evidence-based therapeutic approaches for effective use of cancer immunotherapy in elderly patients. [1]

Immunosenescence and vaccination

An effective medical procedure that has had a significant impact on both healthspan and life expectation of populations is vaccination. [22] Vaccination efficacy depends upon many factors such as age, health status, host genetics, nutritional status, immunological imprinting from previous exposure to infections/ parasites, presence of chronic infections and the vaccine composition. [22]

The elderly is a group at higher risk of developing side effects or a poorer immunological effects from the vaccine administration due to immunosenescence. [22] There is a high risk for

development of potentially lethal infections, longer recovery and long-lasting sequelae. [22] The alteration in controlling autoimmunity in the elderly has a negative impact in the safety of vaccination in this population although considered until now an acceptable risk. [22]

Vaccination protocols have been designed and live-vaccines are rarely used in the elderly and, due to the less effective response to non-live-vaccines, the use of immune-boosters is mandatory. [22]

Influenza vaccine administered to older adults generates around 10-30% seroconversion (against 50-76% in younger individuals) with a less varied antibody repertoire against the virus. [22] After influenza vaccine administration, different patterns of clonal expansion of T-helper (Th) cells have been observed, with an impairment in Th1/Th2 cytokine production with impact in the antibody response efficacy. [22] Also NK sub-type cells show differences between younger and older people after administration of influenza vaccine.

A strategy adopted to improve the response rates of influenza vaccination in the elderly is the administration of high-doses, adjuvated vaccines and recombinant vaccines in this population. [22] Microbiota can have a role as a natural adjuvant for the response to influenza vaccine through administration of probiotics, due to its participation in the immunity modulation [22]. (see chapter 1.7.9)

1.7.9. Microbiota

The gut microbiome has emerged as an important immune modulator which composition influences diseases, such as cancer, autoimmune pathologies, metabolic, neurologic and cardiovascular, determining the overall health status. [22] Its composition it is highly influenced by diet, drugs intake, health status and lifestyle which supports the changes observed in older adult gut microbiome composition (see Table 1.3), when compared with younger people, whom tend to have a poorer diet, high medicines consumption and several comorbidities. [22]

Microbiota and their metabolites can modulate immune cells (example, T cells and B cells differentiation through stimulation of TLR-5 signaling and IL-1B and IL-6 production) and cytokines through epigenetic modifications [22] with particular evidence for the inflammation modulation by the short-chain fatty acids (SCFAs) [21] [22] produced by the gut microbiome from undigested complex carbohydrates or the polysaccharide A (PSA) microbial components. [21] SCFAs are less produced by the elderly microbiota with impact in their inflammation reduction by directly inhibition of TNF-α, IL-6 and NO production, direct promotion of IL-10 or indirect mechanism through insulin response modulation. [22] Also, their role in modulation of cancer cell proliferation and response to drugs in colon cancer is impacted. [22]

Microbiota provides substrates for epigenetic enzymatic reactions, such as folate and choline, and chronically generates antigens such as cellular debris, oxidatively modified

proteins, modified DNA and cancer-related antigens which maintain the basal activation of innate immune cells. [21]

The low-grade inflammation present in the elderly changes the gastrointestinal tract barriers allowing gut microbiota-related substances to be found in the circulation and tissues [21], situation named 'leaky gut' [22]. The causes for this phenomenon are not-modifiable changes in smooth enteric muscle or the enteric neural system but also life-style associated causes such as drug consumption or diet changes motivated by changes in the hunger control centers in the brain. [22] Proton-pump-inhibitors reducing chloride production in stomach favor the bacterial overgrowth, antibiotics, non-steroid anti-inflammatory drugs and steroids are some drug classes with high impact in microbiota composition changes. [22]

Table 1.3 Gut microbiome in the elderly [22]

Microbial Species	Effect
Actinobacteria	Improves response to oral vaccination
Proteobacteria	Low cellular and humoral response to vaccination, quite common in centenarians
Firmicutes	Improves response to oral vaccination; most common in the "oldest old"
Bacteroidetes	Low humoral response to oral vaccination
Enterococcus faecalis	Inflammatory effect through ROS production; increases risk of epithelial damage
Clostridium septicum	Inflammatory effect; increases risk of infectious complications
B. fragilis	Stimulates T-reg differentiation; overall anti-inflammatory effect
B. fragilis enterotoxigenic	Stimulates Th-17 differentiation (tumorigenic in mice)
Bifidobacter spp.	Promotes gut homeostasis through competition with pathogens; anti-inflammatory effects

1.7.10. Frailty

The novel approach to frailty, a phenomenon that occurs with aging, is a multidimensional model characterized by the loss of harmonic interaction between multiple dimensions that lead to homeostatic instability. [23] Frailty is characterized by a decline in functioning of multiple physiological systems with elevated vulnerability to stressors, having a high risk to adverse health outcomes (example, cardiovascular disease, depression) that lead to reduction in life quality, hospitalizations, falls, institutionalization and death. [23]

The clinical assessment of frailty has now started to follow a model of three overlapping dimensions: the inner biological mechanism (mitochondrial dysfunctions, oxidative stress, DNA damage, telomere shortening, defective autophagy, DNA methylation, stem cell exhaustion), the intermediate potential physiopathological mechanisms (chronic low grade inflammation, neurodegeneration, anabolic deficiency, energetic imbalance, reduced protein synthesis) and the external dimension expressing the clinical outcome and manifestations of frailty (functional

deficits in daily living activities, reduced mobility, cognitive impairment, malnutrition, sarcopenia, multimorbidity, polypharmacy, geriatric syndromes). [23]

1.7.11. Pharmacokinetics and pharmacodynamics

The pharmacokinetics effect of age-related changes in physiology is dependent on some factors such as drug characteristics, elimination mechanism, co-morbidities and drug-drug interactions in usual polypharmacy of the elderly. [24]

Absorption of orally administered drugs can be affected by gastric acidity, transit time, permeability and first-pass metabolism. [24] Atrophic gastritis and acid suppressive medications are the main causes of reduced stomach acid concentrations (increased gastric pH) from which it may result a reduced absorption of weakly basic drugs (examples, ketoconazole, ampicillin esters and iron compounds) and an enhanced absorption of weakly acidic drugs. [24] The reduction in gastrointestinal motility (slowed gastric emptying, decreased peristalsis and slowing of colonic transport- longer transit time) might increase total absorption of poorly soluble drugs, delayed absorption of highly soluble drugs (reduction in maximum concentration but no change in total absorption), slow-release formulations might have a higher absorption. [24] Drugs passive permeability does not seem to be affected by old age (examples, penicillin, diazepam, metronidazole) but active transport reduces absorption (examples, glucose, calcium, vitamin B12). [24] The first-pass metabolism may be reduced in aging due to reduction in liver blood flow and mass, leading to increased bioavailability of drugs that undergo an extensive first-pass metabolism (examples, nifedipine, labetalol, verapamil). [24]

Although there is age-related change in body composition (reduction in total body water, reduction in muscle mass and relative increase in body fat) and some reduction in plasma protein binding these are not considered to have a clinically relevant impact in drug distribution. [24]

The drugs metabolism is affected by hepatic blood flow which is reduced, having an impact in medications with high extractions ratios, reducing the clearance (examples, amitriptyline, fentanyl, imipramine, levodopa, metoprolol, morphine, verapamil). [24] Phase I metabolism is reduced, mainly due to reduced blood flow and liver size rather than by reduction in the expression or activity of CYP enzymes (examples, ibuprofen, warfarin, temazepam), also due to decreased capacity of drug transfer into hepatocytes of large or protein bound molecules and transfer of oxygen. [24] Phase II metabolism has no age-related change. [24]

Elimination is done mainly by the kidneys and it is known that several comorbidities (example, diabetes) and cardiovascular risk factors negatively affect the renal function in older age, also the use of nephrotoxic drugs (nonsteroidal anti-inflammatory drugs). [24] There is a high interindividual variability in older adults, so medicines with renal excretion must be closely monitored guided by individual patient's GFR. [24]

Drug pharmacodynamics is more difficult to study than pharmacokinetics but it is known that aging originates a decreased number of brain synapses affecting downward receptor signaling (high sensitivity to benzodiazepines, propofol and opioids), neuromuscular junction changes (impact in neuromuscular blocking agents), impaired cardiovascular response to hypotensive side effect of anesthesia, frailty impact in drugs acting in central nervous system due to inability to cope with rapid changes and external stressors. [25]

2. Clinical trials legislation

The United Nations prospections for year 2050 demographic data presented in the first chapter has shown an increase in life expectancy, with population reaching around 85 years in Europe and 80 years in World, coupled with 28% of European population and 16% of World population being aged 65+. Additionally, the rate of ages 20-64 to 65+ is expected to have an important decrease (due to birth rate decline) leading to the existence of less active age people to give support to the elderly (1.9 in Europe; 3.5 in World).

Having present all the aging changes at cellular and physiologic level and the diseases age-related also previously described, it is expectable that these demographic data should stimulate the improvement of the health care provided for the elderly, despite all additional efforts made by science to delay as much as possible these aging consequences, providing also for an increase in the healthy lifespan.

As part of the health care system, it is crucial to ensure that the development of medicines for human use is adequate for the geriatric patient characteristics and needs (such as, physical and/or mental disability, prevalent diseases) while safety and efficacy in this population group is also assured. Clinical trials are a key tool to reach these goals.

2.1. European Union

European Union has adopted a set of clinical trials legislation and guidelines which are published in the official website Eudralex volume 10 of the publication "The rules governing medicinal products in the European Union" and in the European Medicines Agency (EMA) official website. These should be complemented with the guidance provided in volume 4 part I (chapter 2 and 6) and part II, concerning good manufacturing practices.

Table 2.1 Main clinical trials legislation in European Union

Directive 2001/83/EC, 6th November (Annex 1)	Community code relating to medicinal products for human use
Directive 2001/20/EC, 4th April	Approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use To be repealed by Regulation (EU) No 536/2014, 16th April
Directive 2003/94/EC, 8th October 2003	Principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use

	To be repealed by Commission delegated Regulation (EU) 2017/1569, 23rd May 2017 upon application of Regulation (EU) No 536/2014, 16th April
Guideline ENTR/F/2/AM/an D(2010) 3374 (Annex 13 to Eudralex vol 4)	Manufacture of Investigational Medicinal Products To be repealed by Detailed Commission guidelines No C(2017) 8179, upon application of Regulation (EU) No 536/2014, 16th April
Guideline 2010/C 82/01	Detailed guidance on the request to the competent authorities for authorization of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1) To be repealed by Regulation (EU) No 536/2014, 16th April - Annex I and II
Guideline 2011/C 172/01	Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3') To be repealed by Regulation (EU) No 536/2014, 16th April - Annex III
Regulation (EU) No 536/2014, 16th April	Clinical trials on medicinal products for human use Repealing Directive 2001/20/EC, application planned for January 2022
Commission delegated Regulation (EU) 2017/1569, 23rd May 2017	Principles of and guidelines for good manufacturing practice for investigational medicinal products for human use and arrangements for inspections Repealing 'Directive 2003/94/EC, 8th October 2003 upon application of Regulation (EU) No 536/2014, 16th April
Detailed Commission guidelines No C(2017) 8179 (Annex 13 to Eudralex vol 4)	Detailed Commission guidelines on good manufacturing practice for investigational medicinal products for human use Repealing ENTR/F/2/AM/an D(2010) 3374 Manufacture of Investigational Medicinal Products, upon application of Regulation (EU) No 536/2014, 16th April
Directive 2005/28/EC, 8th April 2005	Principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the

	requirements for authorization of the manufacturing or importation of such products
Commission Implementing Regulation (EU) 2017/556, 24th March 2017	Detailed arrangements for good clinical practice inspection procedures

2.1.1. Marketing authorization for medicinal products (MA)

Directive 2001/83/EC is the community code relating to medicinal products for human use intended to be placed on the market in Member States, prepared industrially or manufactured by a method involving an industrial process. [26] Besides the mandatory manufacturing authorization issued by the competent authority of the Member State, no medicinal product may be placed on the market of a Member State unless a MA has been issued by the competent authorities of that Member State in accordance with the Directive 2001/83/EC or an authorization has been granted in accordance with Regulation (EC) No 726/2004 (procedures for the authorization and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency), read in conjunction with Regulation (EC) No 1901/2006 (medicinal products for pediatric use) and Regulation (EC) No 1394/2007 (advanced therapy medicinal products). [26]

To obtain an authorization to place a medicinal product on the market, an application is made to the competent authority of the Member State concerned and the MA may only be granted to an applicant established in the Community. [26]

The application for MA should be composed, among others and with described exceptions, by the results of clinical trials and, if applicable, a statement to the effect that clinical trials carried out outside the European Union meet the ethical requirements of Directive 2001/20/EC. [26]

Annex 1 to Directive 2001/83/EC lists in detail the standard MA dossier requirements and it is divided in four different parts: [26]

- Part I describes the standardized information;
- Part II provides derogations for medicines with specific applications (well-established medicinal use, essentially similar products, fixed combinations, similar biological products, exceptional circumstances and mixed applications (part bibliographic and part own studies));
- Part III for particular application requirements (biological medicinal products- plasma and vaccine-, radiopharmaceuticals, homeopathic medicinal products, herbal medicinal products and orphan medicinal products);

- Part IV for advanced therapy medicinal products (gene therapy, cell therapy, xenogeneic transplantation medicinal products).

2.1.2. Clinical trial information in MA

In the dossier information, the module 5 contains the clinical study reports concerning the following matters: [26]

- biopharmaceutical studies (bioavailability, comparative bioavailability, bioequivalence, in vitro- in vivo correlation, bioanalytical and analytical methods);
- pharmacokinetics studies using human biomaterials (plasma protein binding, hepatic metabolism and interaction, use of other human biomaterials);
- human pharmacokinetic studies (healthy subjects pharmacokinetics and initial tolerability, patient pharmacokinetics and initial tolerability, intrinsic factor pharmacokinetics, extrinsic factor pharmacokinetics, population pharmacokinetics);
- human pharmacodynamic (healthy subject pharmacodynamic and pharmacokinetics/ pharmacodynamic, patient pharmacodynamic and pharmacokinetics/ pharmacodynamic);
- efficacy and safety studies (controlled clinical studies pertinent to the claimed indication, uncontrolled clinical studies, analyses of data from more than one study including any formal integrated analyses, meta-analyses and bridging analyses);

Clinical trials must always be preceded by adequate non-clinical pharmacological and toxicological tests. [26] In general, controlled randomized clinical trials are performed versus placebo and/or versus an established medicinal product of proven therapeutic value. Any other design needs proper justification. [26] Bias must be avoided by using methods such as randomization and blinding. [26]

It is required that the results of all clinical trials should be communicated, both favorable and unfavorable. [26]

The investigator must be provided with a brochure containing all the relevant information known prior to the onset of a clinical trial (chemical, pharmaceutical and biological data, toxicological, pharmacokinetic and pharmacodynamic data in animals and the results of earlier clinical trials), with adequate data to justify the nature, scale and duration of the proposed trial; the complete pharmacological and the toxicological reports be provided on request. [26] For materials of human or animal origin, it must be assured safety from transmission of infectious agents prior to the commencement of the trial. [26]

The clinical trial documentation must contain sufficient detail to allow an objective evaluation, such as: [26]

- protocol: rationale, objectives, statistical design, methodology, conditions for execution and management, details of the investigational medicinal product used; number and reasons for inclusion of patients; measures taken to avoid bias (methods of randomization);
- audit certificate(s);
- list of investigator(s), Member State where the trial was carried out, each patient information, including case report forms on each trial subject;
- final report signed by the investigator(s).

For each trial, it should be reported the following clinical observations: [26]

- number and sex of subjects treated;
- selection and age-distribution of the groups of patients being investigated and the comparative tests;
- number of patients that withdrawn prematurely from the trials and the reasons for such withdrawal;
- where the controlled trials were carried out;
- whether the control group received no treatment, received a placebo, received another medicinal product of known effect, received treatment other than therapy using medicinal products;
- frequency of observed adverse reactions;
- details concerning patients who may be at increased risk, such as, elderly people, children, women during pregnancy or menstruation, or whose physiological or pathological condition requires special consideration;
- parameters or evaluation criteria of efficacy and the results in terms of these parameters;
- statistical evaluation of the results when this is part of the design of the trials and the variable factors involved;
- any signs of habituation, addiction or difficulty in weaning patients from the medicinal product;
- any interactions that have been observed with other medicinal products administered concomitantly;
- criteria determining exclusion of certain patients from the trials;
- any deaths which occurred during the trial or within the follow-up period.

The requirements for clinical trials concerning a new combination of medicinal substances must be identical to those required for new medicinal products and must support the safety and efficacy of the combination. [26]

Total or partial omission of data must be explained. [26]

If unexpected results occur during the trials, further preclinical toxicological and pharmacological tests must be undertaken and reviewed. [26]

If the medicinal product is intended for long-term administration, it must be given information on any modification of the pharmacological action following repeated administration, and the establishment of the long-term dosage. [26]

The review of the safety data should follow guidelines published by the Commission, with particular attention to events resulting in changes of dose or need for concomitant medication, serious adverse events, events resulting in withdrawal, and deaths. [26] Any patients or patient groups at increased risk need to be identified and a particular attention to be paid to the potentially vulnerable patients who may be present in small numbers, such as, children, pregnant women, frail elderly, people with marked abnormalities of metabolism or excretion etc. [26] It should be described the implication of the safety evaluation for the possible uses of the medicinal product. [26]

The conclusions of the study must include an opinion on the safety of the product under normal conditions of use, its tolerance, its efficacy and any useful information relating to indications and contra-indications, dosage and average duration of treatment, any special precautions to be taken during treatment and the clinical symptoms of over dosage. [26]

Part II of the MA application includes module 5 with the requirements for medicines with specific applications: [26]

- well-established medicinal use, it is accepted detailed scientific bibliography with non-clinical and clinical characteristics;
- essentially similar medicinal products, outside described exceptions, it is accepted cross reference of the original marketing module 5 if allowed by the original MA holder;
- similar biological medicinal products, if the information required in the case of essentially similar products (generics) does not permit the demonstration of the similar nature of two biological medicinal products, the clinical profile shall be provided;
- fixed combination medicinal products (new medicinal products made of at least two active substances not previously authorized as a fixed combination medicinal product), requires a full module 5;
- if the medicinal product is intended for a different therapeutic use, a different pharmaceutical form, administration by different routes or in different doses or with a different posology, appropriate clinical trials must be provided.

Specific and extensive module 5 requirements are set up for all advanced therapy medicinal products in Part IV of MA application, which are additional to requirements defined in Part I. [26]

2.1.3. Regulatory requirements for clinical trials

Currently, all clinical trials conducted within the European Community must comply with the requirements of Directive 2001/20/EC of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. [26] During the assessment of a clinical trial application, clinical trials conducted outside the European Community related to medicinal products intended to be used in the European Community, should have been designed, implemented and reported on what good clinical practice and ethical principles are concerned, on the basis of principles, which are equivalent to the provisions of Directive 2001/20/EC. [26] They should have been carried out in accordance with the ethical principles that are reflected, for example, in the Declaration of Helsinki (see Annex 1). [26] [27]

The good clinical practice is a set of internationally recognized ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects. Compliance with this good practice provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible. [28]

Regulation (EU) No 536/2014 from 16th April applies to all clinical trials conducted in the European Union and it will repeal the Directive 2001/20/EC from 4th April and the national legislation that was put in place to implement the Directive [29]. Its application is planned for January 2022 pending on having the required Clinical Trial Information System (CTIS) fully functional. [30] For the purpose of this text, both codes will be approached as, although not mandatory, it is recommended to take into consideration the aspects considered in the new or updated documents related to the Clinical Trial Regulation and apply them to the clinical trials authorized under the Directive, to the extent possible and in compatibility with the legal framework of the Directive.

There is an updated definition for clinical trial according to Regulation (EU) No 536/2014. *Clinical trial* is 'a clinical study which fulfils any of the following conditions: [29]

(a) the assignment of the subject to a particular therapeutic strategy is decided in advance and does not fall within normal clinical practice of the Member State concerned;

(b) the decision to prescribe the investigational medicinal products is taken together with the decision to include the subject in the clinical study; or

(c) diagnostic or monitoring procedures in addition to normal clinical practice are applied to the subjects.'

being *Clinical study* 'any investigation in relation to humans intended: [29]

(a) to discover or verify the clinical, pharmacological or other pharmacodynamic effects of one or more medicinal products;

(b) to identify any adverse reactions to one or more medicinal products; or

(c) to study the absorption, distribution, metabolism and excretion of one or more medicinal products; with the objective of ascertaining the safety and/or efficacy of those medicinal products.'

and being *Subject* 'an individual who participates in a clinical trial as either a recipient of the investigational medicinal product or a control'. [28] [29]

The investigational medicinal product (IMP) is a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial. [28] [29] It is included in this definition products already with a MA but used or assembled (formulated or packaged) in a way different from the authorized form, used for an unauthorized indication or used to gain further information about the authorized form. [28]

For the manufacture and importation of IMP for human use it is mandatory the holding of an authorization [28] [29], with exception for radiopharmaceuticals [29]. The holder of manufacturing authorization must have permanently at his service a qualified person responsible to assure that each batch is manufactured according to the principles of good manufacturing practice, the product specification file and the existence of the clinical trial authorization in the Member State. [28] [29]

Manufacturing is defined as total and partial manufacture, as well as the various processes of dividing up, packaging and labelling (including blinding). [29] Special provisions are to be applied for the labelling [28] [29], which details are described by good manufacturing guidelines on investigational products, and which must use the official language(s) of the Member State(s) where the trial takes place. [28] Regulation (EU) No 536/2014 also includes annex VI with complete detail of requirements for the labelling. [29]

The product specification file is a crucial element of the pharmaceutical quality system because it contains essential reference documents to ensure the manufacture according to the good manufacturing guidelines and the clinical trial authorization, such as specifications and analytical methods, manufacturing methods, approved label copy, storage and transport conditions, clinical trial authorization, protocol and randomization codes, quality agreements, among others. [31]

Directive 2003/94/EC, 8th October 2003 currently provides the detail of the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use, together with Guideline ENTR/F/2/AM/an D(2010) 3374- Manufacture of Investigational Medicinal Products (annex 13 to Eudralex volume 4), both to be repealed, upon application of the Regulation (EU) No 536/2014, by the Commission delegated Regulation (EU) 2017/1569, 23rd May 2017 and the Detailed Commission guidelines No C(2017) 8179 (update of annex 13 to EudraLex volume 4).

The good manufacturing practice applied to the import and manufacture of IMP is a measure of risk mitigation for the subjects and of variability reduction between batches of the same investigational product to avoid interference with the results of clinical trials on what concerns to quality, safety and efficacy.[31] When compared to authorized medicinal products, there is a higher complexity in the manufacture of investigational medicinal due to lack of fixed routines, variety of clinical trial designs and the packaging designs.[31] Randomization and blinding procedures add an increased risk of product cross-contamination and mix-up.[31] Additionally, authorized products may be used which have been re-packaged or modified in some way.[31] The personnel training and the existence of a highly effective quality system are key factors of success in the application of the good manufacturing principles.[31] Manufacturers and sponsors must cooperate and their responsibilities distribution defined in a technical agreement.[31] The texts give orientations concerning the pharmaceutical quality system, the personnel, the premises and equipment, the documentation, the production, the quality control, the batches release, the outsourced operations, the complaints, the recalls and returns.[31]

Both Directive 2001/20/EC and Regulation (EU) No 536/2014 do not apply to non-interventional trials. These are defined as a study where the medicinal product is prescribed in the usual manner in accordance with the terms of the MA.[28] The assignment of the patient to a particular therapeutic strategy is not decided in advance by a trial protocol but falls within current practice and the prescription of the medicine is clearly separated from the decision to include the patient in the study.[28] No additional diagnostic or monitoring procedures shall be applied to the patients and epidemiological methods shall be used for the analysis of collected data.[28]

Regulation (EU) No 536/2014 introduces the concept of low-interventional clinical trial in which the IMP, excluding placebos, are covered by a MA, meaning that quality, safety and efficacy has already been assessed, reducing the risk to the subject safety.[29] The protocol defines the use of the IMP according to the terms of the MA. The protocol might also define that the product is not used in accordance with the terms of the MA, assuring that it is used evidence- based and supported by published scientific evidence on the safety and efficacy of that product in any of the Member States concerned.[29] It should include high quality data published in scientific journal articles, as well as national, regional or institutional treatment protocols, health technology assessment reports or other appropriate evidence.[29] The intervention must pose only very limited additional risk to the subject compared to normal clinical practice.[29]

The low-intervention clinical trials can be of crucial importance for assessing standard treatments and diagnoses, allowing the therapies optimization and, consequently, contributing to a high level of public health[29]. Their usefulness should be extended to the assessment and

improvement of therapies in population groups with specific characteristics, such as the geriatric population.

As detailed in the beginning of this chapter, the applications for MA of a medicinal product require a dossier related to clinical trials carried out on the product which detailed structure and requirements are described in the Annex 1 to Directive 2001/83/EC.

The clinical trial is responsibility of a sponsor in what concerns to initiation, management and financing and this sponsor can be an individual, a company, institution or organization. [28] [29] The sponsor should monitor the conduct of the clinical trial to ensure the reliability and robustness of the results, the safety of the subjects, the compliance with the rules and supply the investigator with an investigator's brochure. [29] The sponsor should be established in the European Union or have a legal representative or contact person in the Union. [29]

An investigator should be designated to conduct a clinical trial at a clinical trial site. [29]

Both sponsor and investigator have civil and criminal liability and it must be assured a system for subject compensation for any damage suffered resulting from participation in a clinical trial, such as an insurance. [28] [29]

In a clinical trial the rights, safety, dignity and well-being of subjects should be protected and the data generated should be reliable and robust. [29] The interests of the subjects should always take priority over all other interests, such as science and society. [29] The clinical trial can only be initiated if the Ethics Committee and/or the competent authority conclude that the anticipated therapeutic and public health benefits justify the risks and may be continued only if compliance with this requirement is permanently monitored. [28] [29]

To allow for independent control as to whether these principles are adhered to, a clinical trial should be subject to authorization before its commencement and the acquisition of an unique identification number. [28] [29]

Directive 2001/20/EC article 9(8) requires the publication of detailed guidance on the format and contents of the clinical trial authorization application, the documentation to be submitted to support the request on the quality and manufacture of the IMP, any toxicological and pharmacological tests, the protocol and clinical information on the IMP including the investigator's brochure; the presentation and content of any proposed substantial amendment; the declaration of the end of the clinical trial. [28] These requirements were published in the guidelines 2010/C 82/01 'Detailed guidance on the request to the competent authorities for authorization of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1)' where the pediatric investigation plan is mentioned as well as justification for participation of special populations as minors. [32] No detailed requirements are defined for clinical trial authorization application specific for geriatric population.

Regulation (EU) No 536/2014 includes annex I with complete detail of requirements for the clinical trial authorization initial application dossier and annex II for submission of substantial modification (a change to any aspect of the clinical trial which is made after notification of a decision which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial [29]). Components detailed in the annex I are: introduction and general principles; cover letter; EU application form; protocol; investigator's brochure; documentation related to good manufacturing practice for the IMP; IMP dossier; auxiliary medicinal product dossier; scientific advice and pediatric investigation plan; content of the labelling; recruitment arrangements; subject information, informed consent form and procedure; suitability of the investigator; suitability of the facilities; proof of insurance cover; financial; proof of fee payment; proof of data treatment according to EU law. [29] No detailed requirements specific for geriatric population are defined for clinical trial authorization application. In the introduction, there is a protocol general requirement for justification of an age group allocation or exclusion from the trial, the indication of the need to study population representing the patients likely to use the medicine, and the need to improve the treatments available for special group (such as the elderly) including requirements in the protocol related to their specific characteristics. [29]

The authorization for the clinical trial is issued after favorable opinion from Ethics Committee and no objection from the Competent Authority. [28] [29] It must be assured that, for the application assessment, the necessary expertise is available, with a reasonable number of persons who collectively have the necessary qualifications and experience. [29] A specific expertise should be considered when assessing clinical trials involving subjects in emergency situations, minors, incapacitated subjects, pregnant and breastfeeding women and, where appropriate, other identified specific population groups, such as elderly people or people suffering from rare and ultra- rare diseases. [29] The assessment people must be independent from the sponsor, the clinical trial site, and the investigators involved, as well as free from any other undue influence. [29]

The ethics committee is an independent body established in a Member State, consisting of healthcare professionals and non-medical members, whose responsibility it is to protect the rights, safety and wellbeing of human subjects involved in a trial and to provide public assurance of that protection, by, among other things, expressing an opinion on the relevance of the clinical trial and trial design, the evaluation made on the benefits and risks, the trial protocol, the suitability of the investigators and the adequacy of facilities, and on the methods and documents to be used to inform trial subjects and obtain their informed consent. [28] [33] The laypersons to be involved should be patients or patients' organizations. [29] Following article 8 of Directive 2001/20/EC it was published detailed guidance on the format and documentation to submit an application to an ethics committee (ENTR/CT 2). [33]

The protection of people is a constant concern along the text of Directive 2001/20/EC and supported to be upheld by Regulation (EU) No 536/2014: [28] [29]

- mention to 1996 version of Helsinki Declaration (see Annex 1) and obligation of informed consent;
- implementation of good clinical practice;
- inspection of compliance with good clinical practice, conducted by the competent authority of the Member State concerned and coordinated by EMA, and generation of good clinical trial data, including justification for the involvement of human subjects;
- requirement of the special attention to be paid to the incapable of giving consent (by matters of age, such as children, or health, such, as persons with dementia);
- protection of health by avoiding obsolete or repeated testing;
- medical care provided by qualified medical doctor;
- existence of an Ethics Committee;
- monitoring of adverse reactions to assure the immediate cessation of any clinical trial in which there is an unacceptable level of risk.

Directive 2001/20/EC Article 3 (´Protection of clinical trials subjects'), Article 4 ('Clinical trials on minors'), Article 5 ('Clinical trials on incapacitated adults not able to give informed legal consent') and Article 6 ('Ethics Committee') are all dedicated to the people's protection, giving attention to informed consent (by the subject or his legal representative), physical and mental integrity, right to withdraw the trial, insurance, evaluation of the anticipated benefits and risks. [28]

Regulation (EU) No 536/2014 keeps these requirements (Article 10 'Specific considerations for vulnerable populations'; Article 31 'Clinical trial on incapacitated subjects'; Article 32 'Clinical trial on minors') having additional protective measures in Article 33 'Clinical trials on pregnant or breastfeeding women' and Article 34 'Additional national measure' intended for people who are in a situation of subordination (example, persons performing mandatory military service) or factual dependency (example, due to state of health). [29] Article 10, which is dedicated to vulnerable populations, provides for minors, for incapacitated subjects, for pregnant or breastfeeding women and, in general terms, for any other specific groups. [29] There is neither specific mention to special care for geriatric population that might participate in clinical trials nor an assumption that geriatric clinical trials happen.

The informed consent is the decision by the subject who participates in the clinical trial (taking the investigational medicinal product or acting as control) to take part in a clinical trial. [28] [29] It has to be taken freely after being duly informed of its nature, significance, implications and risks and appropriately documented, by any person capable of giving consent or, where the person is not capable of giving consent- such as minor or incapacitated subjects-, an

authorization by his or her legal representative.[28][29] Before giving the informed consent, the potential subject should receive information in an interview using a language easily understandable and have the opportunity to ask questions at any moment.[29] Also, adequate time should be provided for the subject to consider the decision to be taken.[29] The informed consent should be written, dated and signed but, if the person concerned is unable to write, it can be an oral consent in the presence of at least one witness may be given in exceptional cases, as provided for in national legislation.[28] [29] Clear rules should be taken for informed consent in emergency situations such as sudden life- threatening medical conditions necessitating immediate medical intervention (example, stroke) for which it is not possible to obtain it previously to the inclusion into an ongoing trial, but to be provided as soon as possible.[29]

It should be notified the start and the end of the clinical trial and the results should be reported, the latest, within one year from the end of the clinical trial or six months in the case of pediatrics clinical trial.[29][32]

The start of the clinical trial corresponds to the first act of recruiting a potential subject and the clinical trial ends with the last visit of the last subject.[29] The information generated in a clinical trial should be recorded, handled and stored adequately for the purpose of ensuring subject rights and safety, the robustness and reliability of the data generated, accurate reporting and interpretation, effective monitoring by the sponsor and effective inspection by Member States.[29]

Guideline 2010/C 82/01 requested by Directive 2001/20/EC defines the requirement of a clinical trial summary report as part of the trial end notification.[32]

Regulation (EU) No 536/2014 includes annex IV with complete detail of requirements for the summary content of the clinical trial results: clinical trial information (if pediatric, regulatory details); subject disposition; baseline characteristics (where age is part of it); end points; adverse events; additional information (example, substantial modification).[29] No detailed requirements are defined for geriatric population. There is annex V with the detail requirements for the clinical trial summary results for laypersons.[29]

There is a known general underreporting of clinical trial results (around 31%, April 2019 data) by the sponsors and a selective reporting of trials with positive outcome, which might impact the scientific efficiency of clinical research and have a negative public health effect due to underreporting of unfavorable results.[34] A joint letter was issued in 2019 by the European Commission, EMA and Heads of Medicines Agencies in order to apply for the compliance with requirements for publishing clinical trial summary results.[34]

All serious adverse events need to be informed by the investigator to the sponsor and the records maintained in case submission is requested to the Member State.[28] As for suspected serious adverse reactions, all relevant information should be recorded and reported

by the sponsor as soon as possible to competent authorities in all Member States concerned and to the Ethics Committee. [28] Regulation (EU) No 536/2014 changes the flow of notification of suspected unexpected serious adverse reactions, to be done first to EMA, which will forward it to Member States for assessment. [29] There is an annual reporting of all suspected serious adverse reactions and the subject's safety. [28] [29] Directive 2001/20/EC defines 'serious adverse event or serious adverse reaction' as 'any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect' [28]. This definition is split and updated in Regulation (EU) No 536/2014, with main change in the 'unexpected serious adverse reaction' designation, and which is now defined as 'a serious adverse reaction, the nature, severity or outcome of which is not consistent with the reference safety information'. [29] For the evaluation of benefit-risks balance other events need to be timely reported, such as an increase in the rate of occurrence of expected serious adverse reactions which may be clinically important, a significant hazard to the patient population (example, lack of efficacy of a medicinal product), or a major safety finding from a newly completed animal study (example, carcinogenicity). [29]

To comply with article 18 of Directive 2001/20/EC, it was established the guidelines 2011/C 172/01 'Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3')' and Regulation (EU) No 536/2014 includes in annex III complete detail of requirements for the safety reporting. Both have details on how to report serious adverse events by the investigator to the sponsor, suspected unexpected serious adverse reactions by the sponsor to the EMA and the annual safety reporting by the sponsor. [29] [35]

Directive 2001/20/EC describes a rational for testing children in clinical trials based on the significant clinical value for those children of the concerned medicinal product, to have scientific information to improve the treatment available. [28] Children are considered a vulnerable population, with developmental, physiological and psychological differences from adults, which make age- and development-related research important for their benefit. [28] No mention is made in Directive 2001/20/EC for the geriatric population in these same terms and needs as an age group with specific characteristics, already detailed in chapter 1, which has an influence on the suitable of the treatments studied in adult population.

Regulation (EU) No 536/2014 article 32 details the care to be taken on 'Clinical trial on minors' but, although there is a reference to the elderly in the Regulation's, concerning the need to improve the treatments available for vulnerable groups through the appropriate study of medicines specific for these groups (meaning the inclusion in the trial of the subjects that represent population groups- like age groups- that are likely to use the investigated medicine), and the requirement to make justification on the exclusion or underrepresentation of an age

group in a clinical trial, there is no provision specific for clinical trials with geriatric patients in any article. [29]

It is stated in Directive 2001/20/EC the need to set up an European clinical trial database to allow the share of information between the several Member States as well as harmonization of rules and requirements and simplification of administrative acts to improve efficacy. [28] Following this requirement, it was established the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) which is the European database for all interventional clinical trials on medicinal products authorized in the European Union (EEA) and outside the EU/EEA if they are part of a Pediatric Investigation Plan (PIP) from 1 May 2004 onwards. [36]

2.1.4. Advantages of Regulation (EU) No 536/2014 application

The application of the Regulation (EU) No 536/2014 will simplify the clinical trial application dossier approval to obtain an authorization and harmonize the procedure among Member States of European Union by the submission of one application dossier to all Member States through a single submission portal. [29] This simplification is of major importance as the future clinical trials, following the scientific development, will target more specific patient populations for which will be needed the involvement of several or all Member States to obtain sufficient patient number. [29] The submission through the single portal of clinical trials carried out in a single Member State will contribute for the European clinical research [29], increasing the transparency of trial information as the trial authorization, conduct and results carried out in EU will be publicly available [30]. Efficiency of all trials in Europe will be increased avoiding unnecessary duplication or repetition of unsuccessful trials. [30]

There are other advantages for sponsors and investigators such as the legal form of a Regulation giving direct provisions to all Member States (harmonizing the process of clinical trials taking place in several Member States) and harmonization of safety reporting and the labelling of investigational medicinal products. [29] As for the definitions, it is introduced the broader concept of ‘clinical study’, described above, which includes clinical trials as a study category defined on the basis of specific criteria and allows its differentiation from the non-interventional studies. [29]

Although the Regulation entered in force in 2014, the timing of its application will be six months after the confirmation by European Commission of the full functionality of the Clinical Trials Information System (CTIS) through an independent audit. [30] This CTIS will contain the centralized EU portal and database for clinical trials and, at current date, CTIS is planned to go live by 31 January 2022. [30] The authorization and oversight of clinical trials remains the responsibility of Member States, with EMA managing CTIS and supervising content publication on the public website. [30]

2.1.5. Regulatory for good clinical practices

Directive 2005/28/EC, 8th April was adopted to comply with Article 1(3) for good clinical practice, Article 13(1) for manufacturing and importation authorization of investigational products and Article 15(5) for clinical trials documentation and clinical trails' inspection related requirements from Directive 2001/20/EC. Nevertheless, the chapter related with good clinical practice is short and lack detailed guidance.

The members of International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) have set up guidelines on good clinical practice which are internationally accepted as a standard for designing, conducting, recording and reporting clinical trials. [29] The ICH guidelines on good clinical practice are consistent with the principles that have their origin in the World Medical Association's Declaration of Helsinki. [29] Directive 2005/28/EC requires ICH guidelines to be considered as the Committee for Medicinal Products for Human Use (CHMP) have agreed on its adoption, being the last version from 2016, EMA/CHMP/ICH/135/1995- Guideline for good clinical practice E6 (R2). Regulation (EU) No 536/2014 also states that the application of its rules is supported in their details by ICH guidelines on good clinical practice if no other specific guidance has been issued by the Commission. [29]

2.1.6. EMA concerns with medicines for geriatric population

European Medicines Agency official website has a page dedicated to geriatric medicines but neither there is binding legislation for medicines to be used by this specific population group, such as it already exists for pediatrics in Regulation (EC) No 1901/2006, from12th December 2006 ordering the mandatory pediatric investigation plan (PIP) related to pediatric clinical trials for the approval of new medicines, since 2008, nor there is an elderly-specific committee such as Pediatric Committee, created to support the development and availability of medicines for children.

Since 1994, the ICH E7 guideline 'Studies in Support of Special Populations: Geriatrics' (see chapter 2.4) was adopted in Europe. Despite the adopted guidelines and the fact that older people are large medicines consumers it has been recognized that this populational group has been excluded from clinical trials. [37] The reasons for this exclusion have been insufficiently justified, using predefined arbitrary upper age limits, other exclusion criteria such as comorbidities or concomitant medications and older people with frailty, which have a higher risk of adverse outcomes. [37]

Due to aging of European population, arose an increased interest on how the specific biological and medical conditions of elderly patients are considered in the development and evaluation of new medicines, a concern about the lack of clinical trials in the very old and the potential health impact due to transfer of the research results into this population. [38]

In 2006, EMA issued an opinion report on how the CHMP guidelines existing at that time were applying the specific requirements for the elderly population defined in the ICH E7, evaluating 10 centrally approved geriatric patients- relevant medicines dossiers for the participation of patients over 65 years in the clinical development and a critical discussion on the adequacy of ICH E7 itself.[38] At the time, it was concluded on general compliance of the relevant guidelines with ICH E7, but with the need to create a section on specific populations/ elderly for data discussion in this patient group, and the reviewed dossiers were also compliant with the number of subjects aged over 65 or the specific studies needed.[38] Nevertheless, recommendations were made for the update of ICH E7 (definition of several age intervals to identify elderly and very elderly, need to clear define frailty, consideration on the difficulty to obtain informed consent from very elderly patients due to lack of will on participation in clinical trials and adjustment of galenic formulations), the need to reinforce with the Companies for recruitment of adequate number of elderly of various ages and to require evaluation of elderly exposure in CHMP report and Summary of Product Characteristics (via the update of the existing templates).[38]

In 2010, EMA adopted ICH E7 questions and answers guideline, to support, clarify and continue to encourage the application of ICH E7.

In 2011, EMA developed a strategy for geriatric medicines with a vision for the research and evaluation of the medicines in an adequate manner for use in this population: reinforcement of the effective application of already existing guideline ICH E7; creation of reflection papers on this matter; creation inside the CHMP of an advisory group on geriatrics; improvement of the availability of information on the use of medicines for older people (assessment report with a section concerning the experience in geriatric population).[39]

The advisory group was created in 2013, named Geriatric Expert Group (GEG), to support the implementation of EMA's Geriatric Medicines Strategy with high level scientific advice on matters related to geriatric medicines and gerontology upon CHMP request: guidelines review, medicines development, pharmacovigilance, training).[40]

A new analysis was made on the scientific guidelines considered relevant to older population under both the first drafting and revision in the period of 2011-2013 for their compliance with ICH E7 requirements (pharmacokinetics, efficacy, safety and drug-drug interaction studies), having EMA and the GEG made the review.[41] 93% of the selected guidelines did not fully complied with ICH E7, receiving comments requesting improvements and, from the ones that, afterwards, followed for approval, 35% did not reflect the comments received.[41]

Following the goals of the strategy, in 2013 it was assessed the quality of the information included in the CHMP Assessment Report and Product Information (Summary of Product Characteristics and Patient Information Leaflet) concerning the compliance with ICH E7

requirements and the presence of tables summarizing older population data. [42] A report was issued for a number of products considered relevant for geriatric population during 2011-2013, resulting in 75% of the products receiving comments requesting improvement of the information, from which 84% accepted to include it in the information document. [42] Besides this instructive documentation review, also several trainings were given. [42]

Continuing the work related with EMA Geriatric Medicines Strategy and the effort to improve older people participation in clinical trials to have representative target population in the studies, specially of those at high risk of adverse outcomes (as are the frail geriatric patients), in 2018 a reflection paper was issued, on physical frailty to support the baseline characterization of patients aged 65 years and older enrolled in clinical trials. [37] This characterization allows the definition of subgroups and a better interpretation of heterogeneous investigation results compared to overall results of the clinical trial (differential treatment efficacy or related adverse events). [37] The 'Short Physical Performance Battery' (SPPB) was decided as the instrument of choice for the baseline characterization of physical frailty because it fulfills the instrument selection defined criteria: prognostic value of disability and mortality- which is the hallmark of physical frailty-, validation status, feasibility of use across all therapeutic areas, ease of use, time required, ease of investigator's training and cost. [37] SPPB assesses lower- extremity function by measures of standing balance, gait speed and ability to rise from a chair, resulting in three classifications: fit patient (normal), pre-frail patient and frail patient. [37] It gives a good prognostic for adverse outcomes of long-term survival, mortality, hospitalization, disability, worsening mobility, falls, decline in function, decline in health and acute medical events. [37] If not possible to use SPPB, the alternative is 'Gait Speed' which has the same prognostic value of disability and mortality, resulting in three classifications: very high risk, high risk and low risk of negative health outcomes. [37] This physical frailty scales allow older population characterization beyond age improving the evaluation of efficacy and safety. [37]

2.2. United States of America

In the United States of America (USA), Food and Drug Administration (FDA) is the entity responsible for ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices. The legislation supporting these responsibilities can be found in the website Electronic Code of Federal Regulation.

There is an extensive and detailed regulation and additional guidance documents related to clinical trials, summarized at the Table 2.2.

Table 2.2 Code of Federal Regulations (CFR) related to medicines for human use and clinical trials

21CFR50	Protection of human subjects
21CFR54	Financial disclosure by clinical investigators
21CFR56	Institutional Review Boards (IRB)
21CFR312	Investigational new drug application
21CFR314	Applications for FDA approval to market a new drug
21CFR210	Current good manufacturing practice in manufacturing, processing, packing or holding of drugs; general
21CFR211	Current good manufacturing practice for finished pharmaceuticals
42CFR11	Clinical trials registration and results information submission

2.2.1. MA and clinical trial information

The marketing of a medicinal product in the USA territory requires approval by FDA after submission of an application. The regulations for this application are defined in Regulation '21CFR314- Applications for FDA approval to market a new drug', composed of eight subparts: [43]

- Subpart A- General provisions: defining the purpose of the regulation to establish an efficient and thorough review of the drug process for the approval or rejection of drugs based on evidence shown for the safety and efficacy and clarifying the definitions used along the regulation;
- Subpart B- Applications: define in detail the information to be submitted, such as the application form, index, summary, five or six technical sections in which it is included the clinical data section and pediatric use section (with pediatric clinical studies data), case report tabulations of patient data, case report forms, drug samples, labelling;
- Subpart C- Abbreviated applications: it applies for drugs that are the same as a listed drug (list of drugs for which abbreviated applications may be submitted), meaning identical active ingredient, dosage form, strength, administration route, conditions of use,
- Subpart D- FDA action on applications and abbreviated applications: defines timeframes and procedures for FDA reception, review and decision on the applications, including communication methods between the parties and dispute resolution;
- Subpart E- Hearing procedures for new drugs: regulates the procedure for the applicant request a hearing in the case of refusal to approve an application by FDA;
- Subpart G- Miscellaneous provisions: regulates several acts such as, new drugs import and export, drug master files, availability for public disclosures of applications data and information, addresses, guidance documents;
- Subpart H- Accelerated approval of new drugs for serious or life-threatening illnesses;
- Subpart I- Approval of new drugs when human efficacy studies are not ethical or feasible.

For the presentation of clinical trial data, subpart B points (d)(5), (6) and (7) define as principal requirements: [43]

- Description and analysis of clinical pharmacology studies of the drug with results comparison between human and animal pharmacology and toxicology data;
- Description and analysis of each controlled clinical study pertinent to a proposed use of the drug, including protocol and the description of statistical analysis used to evaluate the study;
- Description of uncontrolled clinical studies;
- Additional data relevant to safety and effectiveness evaluation obtained by the applicant from other sources;
- Summary of data to evidence the effectiveness for the claimed indications and dosage, presented by gender, age, racial subgroups, and other subgroups such as other pathologies (example, renal failure);
- Summary and updates of all available information on safety information, including animal data, drug to drug interactions, epidemiological studies, etc;
- If there is potential for drug abuse, presentation of related studies;
- Summary of benefits and risks and discussion of why benefits exceed risks under the conditions stated in the labelling;
- Statement that the clinical study was conducted in compliance with the informed consent regulations (part 50);
- Description of the statistical evaluation of the clinical data;
- Pediatric use clinical trial section with similar approach has just described.

It is mandatory, with several detailed waivers, to provide for each application for a new active ingredient, new indication, new dosage form, new dosing regimen or new administration route, data for the assessment of safety and effectiveness of the drug product in the claimed indications in all relevant pediatric subpopulations. The safety and effectiveness assessment must be done using appropriate formulations for each pediatric age group. If there is similitude between adult and pediatric patients, extrapolation can be done from studies in adults added with pharmacokinetics studies in pediatric patients. [43]

There is no regulation in the same terms for geriatric population, apart the mandatory indication of the age groups that participated in the clinical studies when reporting the study data.

2.2.2. Regulatory requirements for clinical trials

Regulation ‘21CFR312- Investigational new drug application (IND)’ includes procedures and requirements for the submission, review and use of investigational new drugs for the

purpose of conducting clinical investigations of that drug.[44] If compliant with this regulation, the IND is exempt from the premarketing approval requirements that, otherwise, are applicable.[44] It is set the requirements that exempt drug products lawfully marketed in the USA from complying with this regulation when used in clinical investigation.[44]

Investigational drug used in more than phase 1 of clinical study must comply with Regulation 21CFR211 'Current good manufacturing practice for finished pharmaceuticals'.[45]

'Clinical investigation' is defined as 'any experiment in which a drug is administered or dispensed to, or used involving, one or more human subjects.'[44] For this Regulation, an 'experiment' is 'any use of a drug except for the use of a marketed drug in the course of medical practice'.[44] Investigational new drug means a new drug used in clinical investigation.[44] Subject means a human who participates in an investigation, receiving the investigational new drug or acting as a control, both healthy human or a patient.[44]

The sponsor is defined as an individual, a pharmaceutical company, a governmental agency, an academic institution, a private organization who takes responsibility for and initiates a clinical investigation.[44]

The general responsibilities of the sponsor are the selection of qualified investigators and providing them the proper information, monitor of the investigation to assure compliance with the investigation plan, keep the study records and promptly notify FDA and investigators of new adverse effects or risks for the drug under study.[44]

The sponsor is responsible to only begin a clinical trial and administer an investigational new drug to human subjects after submission of the IND to FDA and after it becomes under effect (thirty days after submission, unless FDA notifies that NDA is put on hold) and if in compliance with all applicable requirements of the Regulations 21CFR312 (IND), 21CFR56 (Institutional Review Boards) and 21CFR50 (protection of human subjects).[44]

The submission of IND can be done for one or more of the three phases of investigation.[44] Investigation phase 1 is designed to obtain information on pharmacokinetics, drug metabolism, pharmacologic effects and adverse reactions at increasing doses, being conducted in 20 to 80 patients or normal volunteer subjects.[44] Phase 2 includes controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication(s) in patients with the disease under study and the short- term side effects.[44] Phase 3 studies include from hundreds to thousands subjects and are intended to obtain additional information on effectiveness and safety to allow the benefit-risk evaluation.[44] FDA will evaluate the assurance of safety and subjects' rights in all three phases, for phase 1 focused on the safety of the study, for phase 2 and 3 it will be additionally assessed the scientific quality of the clinical investigation and the obtention of data with sufficient quality for marketing approval.[44]

Regulation 21CFR312 gives the details on the content and format of the application to be submitted, such as general investigation plan, drug characteristics, protocol details,

information on investigators qualifications, control and manufacture of the drug substance and drug product, labelling, pharmacology and toxicology information, the content of investigators' brochure and plans for assessing pediatric safety and effectiveness. [44] No reference is made for geriatric population.

It is prevented the promotion of the investigational drug for the claims under study. [44]

The sponsor is responsible to review the safety information and to issue IND safety reports to FDA and investigators on potential serious risks. [44] In these serious risks are included serious and unexpected suspected adverse reactions (unexpected reactions are not listed in the investigators' brochure for the drug under investigation, or with different severity from previous observations, or different risk from the description in general investigational plan), findings from other studies, findings from animal or in vitro testing, increased rate of occurrence of serious suspected adverse reactions (suspected reactions have a reasonable possibility to be caused by the drug) and unexpected fatal or life-threatening suspected adverse reaction. [44] For an event to be classified as serious, it should result in death, life-threatening, inpatient hospitalization or prolongation of existing hospitalization, persistent or significant incapacity to conduct normal life functions or congenital anomaly/ birth defect. [44]

The sponsor is responsible to issue annual reports on the progress on the investigation. [44]

The investigator is the person who conducts the clinical investigation and he has responsibility to ensure that the investigation follows the investigational plan and applicable regulations, to obtain informed consent from the subjects participating and to protect their rights, safety and welfare, to control the drugs under investigation, keep the investigation records and follow Regulations 21CFR50 and 21CFR56. [44]

The investigator issues to the sponsor several reports such as, the study progress, safety on serious adverse events, final report of the study, financial. [44]

FDA will use the disclosed information on financial arrangements between the sponsor and the investigator to decide on the existence of bias due to financial interest of the clinical investigator in the outcome of the study. [46] This procedure is detailed in Regulation 21CFR54 'Financial disclosure by clinical investigators'.

FDA can issue an order of clinical hold to delay a proposed clinical investigation or to suspend an ongoing investigation, in which the investigational drug cannot be given to the subjects and no new subjects can be recruited. [44] The grounds for the clinical hold are related to the drug risk (known risks or deficient risk information), the qualification of the clinical investigators, the quality of investigators' brochure, deficient protocol for the investigation, lack of study adequacy or control, existence of other studies strongly suggesting lack of effectiveness, existence of another drug for the same indication with a better potential benefit/risk balance [44]

FDA can issue an order of termination and the sponsor ends all clinical investigations and recall all unused supplies of the drug. This may be based on deficiencies in the IND or in the conduct of the investigation. [44]

FDA can issue an inactive status for the clinical study if no subjects are entered into clinical studies for a period of two or more years or the clinical hold persists for one or more years and it will be terminated if the inactive status persists for five or more years. [44]

FDA can inspect both sponsor and investigators records and reports. [44]

Additional and specific requirements are detailed in Regulation 21CFR312 such as, drugs intended to treat life-threatening and severely- debilitating illnesses, import/ export requirements for the investigational new drug, foreign clinical studies not conducted under an IND, availability for public disclosure of IND data information, expanded access to investigational drugs for treatment use. [44]

Regulation 42CFR11 'Clinical trials registration and results information submission' defines the requirements and procedures for the submission of clinical trial information to be made publicly via ClinicalTrials.gov which is the internet clinical trial registry and results data bank established by the National Library of Medicine. It is mandatory that each clinical trial has a sponsor which is responsible for it and for the execution of requirements in this Regulation. [47] The clinical information submitted should not be false or misleading, being subjected to civil monetary penalties and/or other civil or criminal remedies. [47] The clinical trial register is mandatory, the latest until 21 calendar days after its initiation (date in which the first human subject is enrolled), and it is composed of descriptive information (among others, title, brief summary, design, study phase, primary disease under study, product under study, dates), recruitment information (among others, eligibility criteria, gender, age limits), location and contact information (institution and sponsor) and administrative data (among others, the unique protocol identification number, FDA IND number, human subjects protection review board status). [47] The completion date of the clinical trial refers to the date on which was completed the data collection of the final subject for all the primary outcomes (outcome of the greatest importance specified in the protocol) and the results should be submitted no later than one year after this date. [47] There is an extensive and detailed list of results information to be submitted, such as participant flow (starting and ending number, detail of participant flow arm), demographic and baseline characteristics (arm, population, characteristic measured, measure data, number of participants, age), outcomes and statistical analysis, adverse event information, protocol and statistical analysis plan, administrative information (point of contact for scientific information about the clinical trial results). [47]

2.2.3. Regulatory for good clinical practices

Any clinical investigation on drugs for human use submitted to FDA must comply with Regulation 21CFR56 'Institutional Review Boards' and cannot initiate before revision and written approval by an Institutional Review Board (IRB)- notified to investigators and the institution- in their compliance with the protection of the rights and welfare of the human subjects involved in the investigations (Regulation 21CFR50). [48] This IRB is defined as a committee designated by an institution to approve the initiation of and to conduct periodic review of (at least, once a year) research involving human beings and which committee has to meet the requirements of Regulation 21CFR56. [48] The IRB must be registered in the Unites States Department of Health and Human Services, should be composed of at least five members with varying and adequate background related to the investigation to be evaluated in what concerns to scientific area but also nonscientific, such as knowledge and experience working with vulnerable category of subjects (children, prisoners, pregnant women, mentally disable, etc) and community attitudes. [48] It has to be taken into consideration the composition of the committee in relation of race, gender (both sexes must be represented) and cultural backgrounds to avoid any discriminatory selection. [48] The approval of the research by the IRB requires that investigation complies with: [48]

- Procedures defined to minimize the risk to subjects (the harm or discomfort anticipated in the research is not higher than those encountered in daily life or in routine examination or tests);
- The risks to subjects are reasonable in relation to anticipated benefits (risks and benefits resulting only from the research);
- Equitable selection of subjects for the purpose of the research and consideration for vulnerable populations, in case of children the compliance with Regulation 21CFR50 subpart D; no similar concerns are regulated for geriatric population;
- Informed consent given by each subject or legally authorized representative (according to Regulation 21CFR50);
- If applicable, adequate provisions were taken to monitor data collection to ensure the safety of subjects and protect their privacy and maintain the confidentiality of data.

Any clinical investigation on drugs for human use submitted to FDA must comply with Regulation 21CFR50 'Protection of human subjects' to assure the rights and safety protection of the human subjects involved in the investigation. Part B of Regulation 21CFR50 regulates informed consent which is mandatory to be obtained for each subject before the involvement in any research. [49] The general requirements are the use of understandable language adequate to the subject, sufficient time for the subject consider to participate or not in the research and clear wording in the consent (written or oral) to do not waiver any of the subject's

legal rights or investigator, sponsor or institution from liability for negligence.[49] The informed consent is documented in a written consent form approved by the IRB and signed and dated by the subject or subject's legal representative. It can also be a short form written consent stating that the elements required for informed consent were presented orally (in the presence of a witness) to the subject or the subjects' legal representative.[49] The basic elements of the informed consent are statements related to voluntary participation, the right to withdrawal participation at any time without penalty or losing entitled benefits, the explanation of the research (purpose, duration, identification of experimental procedures), description of foreseen risks and benefits to the subject, alternative procedures or courses of treatment, extent of subject data confidentiality, procedure in case of injury (compensation and medical treatment), contacts.[49] There is an extensive and detailed list of exceptions to the informed consent related to general requirements and for emergency research.[49]

2.2.4. FDA concerns with medicines for geriatric population

Although the mandatory clinical trial Regulations do not present specific requirements for geriatric population in the drug studies, there is guidance in this subject since 1989 when the 'Guideline for the study of drugs likely to be used in the elderly' was issued. It is emphasized that the patients included in clinical studies should reflect the population that will receive the drug when marketed to support that older patients should be included in reasonable numbers in clinical trials for drugs likely to be used in the elderly, although taking care of excluding patients in the impossibility of assuring their safety, ethics and usefulness.[50] The exclusion should not be arbitrary (based on advanced age alone, concomitant illness or medication unless danger results for the patient or misinterpretation of study results) and, when applied in earlier studies, be re-evaluated and, if possible, reverted for Phase 3 studies.[50] The determination if a drug is likely to have significant use in the elderly might be obvious in some diseases like coronary artery disease or senile dementia, for instance, but when the use is not so clear it is advised to search the estimates of the disease prevalence by age or an evaluation of age distribution of other similar drugs.[50] It is stressed that the evaluation of possible differences in dose response between younger and older people should focus on pharmacokinetics which is more frequent and better documented than pharmacodynamic differences, resulting from age-related alone or age-associated conditions such as renal impairment, congestive heart failure or multiple drug therapy.[50] It should be evaluated the effect on pharmacokinetics of renal impairment by the study of drugs that are excreted mainly through renal mechanisms, which will support the dose adjustment.[50] The same applies for hepatic impairment or drugs that affect hepatic metabolism and drug-drug interaction.[50] The pharmacokinetics screening helps to interpret clinical findings in Phase 2 and Phase 3 such as unusual effectiveness or adverse reaction responses.[50]

In 1993, FDA adopted ICH E7 guideline 'Studies in Support of Special Populations: Geriatrics', discouraging the arbitrary maximum age requirements and encouraging the inclusion of participants with concomitant illness and receiving concomitant medications, who are often older adults.[51] (see chapter 2.4)

In 2012, FDA adopted ICH E7 'Questions and answers' guideline clarifying the application of the ICH E7 guideline and emphasizing the inclusion of older patients in clinical trials, especially 75 years or older.[51] (see chapter 2.4)

In 2014, FDA Safety and Innovation Act developed an action plan to enhance the collection and availability of demographic subgroup data, divided into three priorities: improving the completeness and quality of demographic subgroup data collection, reporting and analysis (quality); identifying barriers to subgroup enrollment in clinical trials and employing strategies to encourage greater participation (participation); and making demographic subgroup data more available and transparent (transparency).[52] In this action plan, it is identified the need to review existing guidelines such as ICH E7, or the Integrated Summary Effectiveness to increase attention to demographic analysis in the review of the clinical trials annual reports where tabular data already includes the age of participants.[52] Another action proposed was the update of standardized age categories, grouping older patients in more discrete categories for analysis (65 to 74 years old, or 75 to 84 years old, rather than younger than 65 years old or simply using birth year).[52] It is identified that subgroups of age over 75 years are less likely to be enrolled in clinical trials than the population as a whole and, being a voluntary participation, some barriers might need to be overcome, such as, limited numbers of investigators who can help enroll underrepresented subgroups or who have access to a broader range of patient subgroups; patients and families with negative attitudes about medical research and concerns about risk; patient inconvenience; availability of transportation; geographic location; and insurance status.[52] Additionally, the lower participation by patients over the age of 75 is thought to reflect protocol exclusions, which needs to be worked to improve the enrollment criteria.[52]

In November 2020, the Guidance for Industry 'Enhancing the Diversity of Clinical Trial Populations— Eligibility Criteria, Enrollment Practices, and Trial Designs' has been issued with recommendations to sponsors to support the enrollment of participants with a wide range of baseline characteristics that more accurately reflects the patients likely to use the drug and to allow assessment of the impact of those characteristics on the safety and effectiveness of the study drug, in an effort to increase the participation of groups that continue to be underrepresented in clinical trials, in which geriatric population is an identified part among others.[51]

Despite the efforts made by FDA in the past few decades promoting enrollment practices to execute clinical trials that would better reflect the population most likely to use the drug,

mainly through the broadening of eligibility criteria (characteristics the participants must or must not have to be able to participate in the study, also known as inclusion and exclusion criteria), certain groups, such as geriatric population, continue to be underrepresented. [51] Eligibility criteria is used to exclude people from participating in a trial for whom the risk of an adverse event outweighs both that individual's potential benefit from participating and the importance of the knowledge that may be expected to result. It is recognized that certain exclusions are appropriate when necessary to help protect such individuals. [51] However, some eligibility criteria have become commonly accepted over time or used as a template across trials, sometimes excluding certain populations from trials, such as older adults, without strong clinical or scientific justification. [51] Beyond the eligibility criteria, there are clinical trial design features that impact their enrollment, such as geographic location and physical accessibility difficulties of the study site, frequency of visits to the study site, personal expenses. [51]

In this guidance, advice is given related to broadening the eligibility criteria to increase diversity in enrollment and avoiding unnecessary exclusions from clinical trial (evaluation if existing criteria serve the goal of having a representative sample of population for whom the drug has been developed; evaluation of each exclusion criteria; evaluation if more restrictive exclusion criteria in phase 2 trials can be eliminated or modified in phase 3 trials), in the design of clinical trials (early characterization of drug metabolism and clearance to avoid later exclusions and to allow dose adjustments across different populations; adaptive clinical trial design to allow changes when more data become available assuring the safety participation of a broader population), use of enrichment strategy targeting the enrollment to population more likely to reach the study endpoints. [51] Other recommendations are made to increase the participation of people, such as making trial participation less burdensome (frequency of visits, location of site), education of participants, patient-focused research included in trial design (understanding of their needs), providing cultural competency for clinical investigators to build a trusting relationship with participants, recruitment events, financial concerns. [51]

To be noted that FDA's guidance and adopted ICH guidelines are non-binding, representing FDA's current thinking on the topic, and alternative approaches are allowed if they satisfy the applicable statutes and regulations.

2.3. Japan

In Japan, the National Institute of Health Sciences (NIHS) conducts testing, research, and studies for the proper evaluation of the quality, safety, and efficacy of pharmaceutical products to assure their public benefit. In its site there is an English language summary on the regulatory developed for the pharmaceutical sciences.

Inside the NIHS, the Ministry of Health, Labour, and Welfare (MHLW) (Koseirodosho in Japanese) is responsible for pharmaceutical regulatory affairs, and the Pharmaceutical and Medical Devices Agency (PMDA, KIKO) provides consultation in new drugs clinical trials and conducts approval reviews and surveys on the reliability of the application data. [53] Together they handle a wide range of activities from clinical studies to approval reviews, reviews throughout post-marketing stage, and pharmaceutical safety measures. [53]

The drug marketing approval requires an application form submitted to PMDA and decision taken by the MHLW. [53] Since 2003 it was adopted the common technical document (CTD) from ICH guidelines for the preparation of approval applications, containing the module 5 dedicated to Clinical Study Reports. [53]

Japanese clinical trials adopted, since 1997, into internal Ordinance the ICH E6 Guideline on Good clinical practices (see chapter 2.4) to assure the human rights, safety and reliability on clinical study data. [53] By that time, it is recognized a general need to encourage voluntary participation of human subjects in the clinical studies with some measures taken such as, guidelines to improve the clinical research facilities and equipment, education and training of the clinical research coordinators, definition of rules for efficient subjects recruitment. [53] It was also developed standard operating procedures for the audit and monitoring of the medical institutions that presented clinical practices problems. [53]

In 2005 was established the Council on Efficient Conduct of Clinical Trials with main goals the evaluation and solution proposal for efficient conduct of clinical trials, assuring reliability of the study and safety of the subjects, also applied to investigator initiated clinical trials, and improvement of the institutional review board performance. [53]

From the Council work resulted, in 2008, a review of the Ordinance for Good Clinical Practice in chapters for allocation of investigational products, adverse drug reaction reports and institutional review boards. [53] The sponsor must submit a study protocol to MHLW with nonclinical data supporting the safety and efficacy, other pre-existing human studies, the subject population, among others. [53] Sponsor must assure obtaining the written informed consent from the subjects participating in the trial, the existence of compensation for any health impairment resulting from the study, report to authorities any severe adverse reactions, receive on-site inspection from authorities concerning CGP compliance in case of problems during the study, the issue of an investigator's brochure. [53]

GMP rules were update in 2008, assuring the preparation of the investigational product with a concern for the pharmaceutical consistency, assuring the equivalence between the product used in the trial and the marketed product after approval. [53] Medical institutions performing clinical trials must assure the establishment of an Institutional Review Board to discuss and review the proper and ethical conduct as well as the scientific appropriateness of the clinical trial. [53]

Following ICH guidelines, in 2010, 34 guidelines for medicines clinical evaluation were published. [53]

For pediatric use medicines, considered as orphan in Japan, a problem is recognized regarding the insufficient number of clinical trials performed in children, existing only a few number of medicines that can be used for children, added of insufficient information in the package insert, resulting common the “off-label use” of drugs intended for adults, the use of in-hospital products without adequately verified stability, and the use of individual import drugs for pediatric use. [53] When indications related to off-label use are public knowledge in medicine or pharmacology, these can be requested, without additional clinical trials formal studies, to be added in an application for partial changes. [53]. At present, laws and regulations aimed at drug development and direct promotion of information dissemination in the pediatric field such as those in the EU and United States do not exist in Japan, although ICH E11 Clinical Investigation of Medicinal Products in the Pediatric Population has been adopted in 2000. [53] In March 2006, the Study Group on Pediatric Drug Treatment was established, dedicated to the efficacy and safety of pediatric drug treatment, conduction of surveys on drug prescriptions for pediatric use and to provide information to health professionals for the environmental improvement to adequate pediatric drug treatment. [53]

For medicines with use intended for geriatric patients there is only a short mention on the elderly as part of special populations and a reference for ICH E7 guideline in an extensive list of adopted guidelines. [53]

2.4. ICH

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) works on the harmonization of scientific and technical aspects of the drug registration. [54] This is done with the development of ICH Guidelines by regulatory authorities and pharmaceutical industry working together in scientific consensus and having a commitment by ICH regulators for the implementation of the final guidelines. [54] It started in April 1990 with regulatory founding members from Europe, Japan and the USA and, by November 2020, it had internationally spread to Canada, Switzerland, Brazil, Singapore, Republic of Korea, China, Chinese Taipei and Turkey. [54]

In its website several guidelines can be found related to efficacy of medicines which are concerned with the design, conduct, safety and reporting of clinical trials. This harmonization of clinical trials procedures is important for mutual acceptance of the studies between the members’ regulatory authorities.

Table 2.3 Main ICH Guidelines related to good clinical trials practices

E6 (R2)	Good clinical practice
E8	General considerations for clinical trials
E1	Clinical safety for drugs intended in long-term treatment
E2A	Pharmacovigilance
E3	Clinical study reports
E7	Clinical trials in geriatric populations
E11	Clinical trials in pediatric populations
E9	Statistical principles for clinical trials

2.4.1. Guideline for good clinical practices

ICH E6(R2) guideline on good clinical practice follows the Declaration of Helsinki for the assurance of the rights, safety and well-being of trial subjects and, at the same time, defines an international standard for the scientific quality design, conduction, recording and reporting of clinical trials producing credible data.[55] Its guidance only allows initiation of a clinical trials after evaluation if the anticipated benefits justify the risks, assuring that science and society come after the trial subject interests, which voluntary participation is evidenced by the signature of an informed consent.[55] The key stakeholders designated to assure the compliance with these principles are the Independent Ethics Committee (IEC) or the Institutional Review Board (IRB), the sponsor/ organization and the investigator.

The IRB/IEC protect the rights, safety and well-being of all trials subject by giving the approval/ request for modification/ disapproval/ suspension of previous favorable opinion on the clinical trial after evaluation of a set of documents (trial protocol, informed consent form, written information to be given to the subjects, subject recruitment procedures, investigator's brochure, safety information available, payments and compensations for the subjects) and the investigator's qualification.[55] The IRB/IEC is composed of at least five members, including expertise members, at least one member in nonscientific area and at least one member independent of the trial site.[55] IRB/IEC acts on the initiation of the clinical trials and make periodic review of it.[55] No subject should be admitted in the trial before the IRB/IEC issues a trial written approval.[55]

The sponsor is defined as the entity (individual or institution) which takes responsibility for the initiation, management and financing of the clinical trial.[55] The guideline presents an extensive and detailed list of responsibilities related to quality and risk management (for the systems- example, computerized systems-, and for the clinical trial -example, the trial design), the medical expertise and investigator/ institution selection, data and records handling, financing, the review by IRB/IEC, the investigational product (information on safety and efficacy from nonclinical studies, manufacturing procedures, supply), adverse reaction reporting, monitoring of the trial, reports and communication with the authorities.[55]

The investigator should be adequately qualified and instructed with the investigational product information given by the sponsor (example, trial protocol and the Investigator's Brochure) assuring the same information for his team. [55] The guideline presents an extensive and detailed list of responsibilities related to the medical care to be provided to the subjects, the communication with IRB/IEC, the compliance with the protocol, the control of the investigational product use and storage, the good practices for obtaining informed consent from the subjects (with details on the information to be given to the subject), records and reports. [55]

ICH E6(R2) finishes with instructions for the Investigator's Brochure preparation and a guiding list of the essential documents for the clinical trial conduct. [55]

ICH E6(R2) is currently under review with the aim of: inclusion of different types of study designs; use of new health technologies for the improvement of the trial design, the selection of relevant patient population, the trial documentation; strength the use of risk-based approach to the trial design and conduct; improve the focus on key principles and objectives. [56] The draft of version R3 principles was made available in April 2021.

2.4.2. Guideline for clinical trials in geriatric population

For the elderly, since 1993 it was issued the ICH E7 guideline giving support to the studies in geriatric population to be applied for new molecules, new formulations and new combinations for which is expected an impact in the elderly. [57] In 2010, it was issued a question and answer document to make clarification on the application of the guideline key points.

It is emphasized that geriatric patients respond differently to drug therapy from younger patients (due to age related physiological changes), added that not all potential differences (pharmacokinetics, pharmacodynamics, disease-drug, drug-drug interactions, clinical response) can be predicted from non-geriatric studies, due to higher probability of comorbidities and concomitant therapies (higher risk of adverse effects). [58]

Although the geriatric population is arbitrarily defined as aged 65 years or older, the importance to include in the studies also ranges over 75 years old is highlighted so that upper ages are not cut off from the studies. [57]

The inclusion should be done mainly on Phase 3 (Phase 2 at sponsor's option) with a minimum of 100 patients to allow enough representation for results comparison with younger subjects, although the studies might be exclusively conducted in geriatric. [57] There is a preference for the use of both groups (geriatric and non-geriatric) in the same study for direct comparison of results obtained in similar conditions. [57] If the enrollment of the geriatric patients is insufficient, a specific plan to collect data pos-marketing should be discussed during drug development and be presented in the marketing application. [58]

Whenever possible, data from the study should be presented for various age groups (<65, 65-74, 75-84, >85) to allow comparison of treatment effect consistency and safety profile with the non-geriatric group. [58] The most important differences between younger and older patients are identified as pharmacokinetics (due to impairment in excretion via renal/hepatic or drug to drug interactions) and guidance is given for formal pharmacokinetics studies or a screening during Phase 2/ 3 clinical trials. [57] It is also considered pharmacodynamic studies for drugs with important central effects or appearance of young/elderly differences in drug's effectiveness or adverse reaction profile not explained by the pharmacokinetics differences. [57] The recommendation for drug-drug interaction studies are done for drugs with a narrow therapeutic range or if there is an inducer/inhibitor effect in hepatic enzyme for drugs with extensive hepatic metabolism in concomitant therapies. [57]

It is expected to have the information related to geriatric patient population (including limitations) described in the product labelling. [58]

3. Clinical trials and the geriatric patient

3.1. Underrepresentation of geriatric subjects in clinical trials

General underrepresentation of geriatric population on clinical trials is assumed by regulatory authorities of FDA, EU and Japan and validated also by many studies.

This happens despite the existence of the already described legislation requirements for the representation of the target population that will use the approved drug, the justification needed for the exclusion criteria, the existence of specific guidelines (example, ICH E7) for the execution of clinical trials in geriatric patients.

A study from 2020 indicates that around 87,1% of European patients aged 75+ use prescribed medicines.[59] In 2014, it was identified that subject participation in clinical trials for drugs recently approved were of only 57% for ages 65+ and 22% for ages 75+ in clinical trials involving diseases characteristically associated with aging and a drastic decrease to 9% for ages 65+ and 1% for ages 75+ in studies involving more generic diseases.[59] In 2019, an evaluation of FDA approved drugs for the last decade showed that initial approval documents had pharmacokinetics information in an elderly subpopulation for 62% of the drugs but only 42% for safety and 45% for efficacy.[59]

Without the necessary number of geriatric patients represented in the clinical trials it is not possible to evaluate the benefit/risk balance associated with the medicines use in this population group. Data extrapolation from studies in younger adults might not be possible, given the existing lack of guidance in this procedure. There are concerns at public health level, as doctors must decide between an off-label use, with all the risks from the lack of safety and efficacy evidence- which is different from data obtained from the younger due to the aging process as already here presented (and among the elderly, different between healthy or having co-morbidity and concomitant therapeutics)- or not providing the medicine at all. This originates a discrimination in the access by the geriatric patients to therapeutic advances.

Currently, there is no binding law directly making the approval of medicines conditional to the study of the IMP in the geriatric population group.

3.1.1. What are the possible root causes?

Several root causes for the clinical trials underrepresentation of geriatric patients can be identified and are detailed below.

Non-compliance with the legislation/ guidelines by the clinical trial team

The exclusion criteria are allowed as a protection for the subject if there is a reasonable justification for it, like a potential negative benefit/risk or the short scientific advance taken from the study. In practice, it is arbitrarily used for the exclusion of older aged subjects.

There are several characteristics that turn geriatric patients into an undesirable population group in the clinical trials, besides the upper age limit discrimination, which at the same time are the reasons why is crucial to have them represented to obtain data to support evidenced- based medicine practice:

- Comorbidity, concomitant medication and the level of frailty turn them into a vulnerable population group with a higher risk of adverse events occurrence and toxicity;
- These variables originate higher difficulty in the assessment of cause-effect in the efficacy and the safety related to the IMP due to drug-drug interactions and drug-disease interactions;
- Complexity in the data analysis for a correct determination of a safe and effective dosage regimen in the geriatric patient group for the MA. There is a high inter-individual variability related to the aging process and tendency for diseases development which can vary significantly for the same age group/age sub-group.

Impact of clinical trial design in the enrollment acceptance by the geriatric patient

The clinical trial design can act as a burden for elderly patients, originating their refusal in the enrollment:

- Location of the site (distance from home) and installations accessibility;
- Frequency needed for the site visits;
- Disease impact (level of physical/ mental disability);
- Physical and psychological discomforts from the procedures (example, sample collection more- or- less invasive and frequent, pain, cold or heat);

Misfit of the recruitment procedure

- Lack or ineffective clinical trial publicity targeting the elderly;
- Ineffective communication from the investigator for the subject potential benefits from participation in the clinical trial;
- Lack of empathy and trust between investigator and the subject (including the family which has a great impact in the subject acceptance to participate in the clinical trial);
- Poor quality of the patient available information (example, language understandable by the person, adequate letter size in written information);
- Negative perception by the subject of the benefit/risk balance which can be magnified by the discomforts from the trial procedures;
- Unclear understanding or short monetary compensation for the clinical trial participation expenses and the assurance of insurance protection for any occurrence from the clinical trial;
- Unclear understanding or poor follow up to be provided after the trial is ended.

3.1.2. How to improve geriatric patient representation in the clinical trials? Regulatory approach

Binding and geriatric specific regulation related to clinical trials for development of medicines that will be used by geriatric patients should be put in place to provide intervention on the critical steps and subordinating the MA approval to availability of results for the geriatric benefit/risk evaluation and dosage regimen definition.

Some of the below recommendations are preventive measures due to the step in which they are applied (namely, IRB/IEC composition, investigator team composition, evaluation of the protocol, evaluation of the clinical trial execution) since it would still be possible to make the required corrections on time for obtaining the necessary data for the geriatric patients from the study.

1. IRB/IEC members

It should be assured that the members composition of the IRB/IEC evaluating a clinical trial for a medicine to be used by geriatric patients includes expertise in geriatric matters.

2. Protocol

The protocol evaluation before the clinical trial approval should consider:

- **Disease treated by the IMP**: evaluation of the disease prevalence in the geriatric population to be used as an inclusion criterium;
- **IMP**:
    - Pharmacokinetics: existence of data about level of renal or hepatic elimination route and the impact in the geriatric population if using the medicine (usual with their changed pharmacokinetics parameters, physiologic due to aging or to disease) to be used as an inclusion criterium. It might be advisable to execute pharmacokinetics screening during the trial or specific studies to make the necessary dose adjustments;
    - Pharmacotherapeutic class: the level of pre-knowledge on the investigational medicine related to the class of medicine it belongs and the use in geriatric population, to be used as an inclusion criterium;
    - Formulation: evaluate the adequacy of IMP formulation and administration route for use in geriatric patients with physical disability, if there is a probability to be a target group for the use of the medicine under study. This will have impact in the compliance with the treatment and the quality of data obtained from the clinical trial;
- **Exclusion criteria**: detailed evaluation of the described exclusion criteria for geriatric patient to assure they are scientifically and clinically based, with a clear identification of negative potential benefit/risk;

- **Baseline frailty level acceptance**: it should be included in the protocol a definition for the baseline frailty level considered acceptable considering the characteristics of the trial/ IMP (guidance already available in EMA's 'Reflection Paper on physical frailty'), to be used as an inclusion criterium;
- **Demographic data**: clear definition of the subjects 65+ age and 65+age sub-groups to be used as an inclusion criterium. It should be challenged the age of 65+ years for the definition of geriatric population considering the current general health status at that age and life expectancy.
- **Specific needs for any disability compensation**

3. Investigators' team

Evaluation of the investigators' team qualification to assure the existence of experts in geriatric matters.

4. Clinical trial execution

Execution of periodic audits/ inspections by the sponsor, the authorities or the IRB/IEC during the clinical trial execution to assure the compliance with the protocol, including the verification of the identified critical points.

Some activities to be stressed are:

- **Recruitment results**:
    - Evaluation of the demographic data of the subjects enrolled in the clinical trial to assure that 65+ patients and, whenever possible, the age subgroups, were included by the investigator according to what was defined in the protocol;
    - Evaluation if the geriatric group includes subjects until the baseline frailty level that was defined in the protocol, with identification if unnecessary exclusions were performed or not;
- **Treatment compliance**: evaluation if the geriatric group has been receiving the treatment according to the protocol instructions (use of monitoring procedures defined in the protocol);
- **Study results**: evaluation of the data quality and transparency that is being collected.

5. Report and patient information

- Evaluation of the geriatric information in the final report and in the summary of product characteristics. The availability and quality of this information should have an impact in the decision for the medicinal product approval.
- This evaluation should be done by a geriatric committee with adequate expertise.

Supporting non-regulatory measures

1. Clinical trial location:

To overcome the physical and psychological burden for the geriatric patient from travelling (distance from home, transport availability and expenses, frequency) and installations' accessibility, which can impact his acceptance for participating in the study or the dropout rate, it could be evaluated the possibility of:

- Development of a home-based clinical trial in which:
    - the clinical trial team carries out the necessary procedures at the subject home (total or mix);
    - the subject/ support people carry on the follow up procedures and report data;
    - simplification of sample collection plan;
    - portability and easy to use of measurement instruments might be a challenge for engineering development;

A paper defending a paradigm change from hard endpoints to value-based endpoints illustrates the benefits, some feasible technologies already existing and examples on how to implement the concept of home-based clinical trial. [60]

- Long distance follow-up (video or phone consultation), whenever possible;
- Facilitation of transport.

2. Adaptive clinical trial demographic requirements:

- As phase 2 and phase 3 have different goals, it should be defined in the protocol the introduction of geriatric patients in the most convenient timing for both the study and the subject. The exclusion from phase 2 should not prevent the participation in the phase 3;
- Along the study, the acquisition of safety and efficacy data should allow the introduction of geriatric patients from a time point on.

3. Recruitment investigators:

- Assurance of an adequate investigators number and time availability for the recruitment of a more difficult to enroll geriatric group;
- Access to communication skills training to improve the approach to the subject and family (clear and empathetic language, adequate to the people characteristics);

4. Adequate information given to the subject and family to overcome:

- Fears from research environment (emotional burden);
- Unrealistic and negative perception of the risks, with clarification of the positive balance benefit/risk from the subject participation on the study.

3.2. Managing the lack of geriatric patients' clinical trial data

3.2.1. Physiologically based pharmacokinetic (PBPK) modelling and simulation

An approach to obtain missing pharmacokinetic data due to underrepresentation of geriatric subjects in phase 1 studies is the PBPK modelling and simulation. This is defined as 'a mathematical model that simulates the concentration of a drug over time in tissue(s) and blood, by taking into account the rate of the drug's absorption into the body, distribution in tissues, metabolism and excretion on the basis of interplay between physiological, physicochemical and biochemical determinants'. [61] Although PBPK modelling has been mostly used to predict drug-drug interactions and support pediatric and first-in-human selection of initial doses, its use can be developed to generate scientific evidence in other populational groups. [61] This method uses specialized software platforms which needs to be qualified if the obtained data is intended for regulatory purposes. [61]

The PBPK modelling combines three essential information types: [62]

- Body systems data: biological and physiological factors relevant to human body, such as age, weight, cardiac output, tissue volumes, tissue blood flows, enzyme abundance;
- Trial design: dose regimen, duration of study, route of administration, co-medication, study population;
- Drug data: molecular mass, solubility, membrane permeability, metabolism, transporters.

These data support the simulation and preview of PK/PD parameters in virtual populations allowing: [62]

- Mapping inter-individual variability of plasma drug concentrations;
- Use of different administration routes;
- Preview drug concentration in different organs (relation to drug site of action);
- Use of several scenarios, including worst case scenario which would not be tested in clinical trial but could happen in marketed drug.

There are PBPK modelling studies made for parenteral, oral and transdermal drug administration intended for prediction of drug absorption and exposure in the elderly. [62]

The prediction of the differences in systemic drug exposure between young adults and older subjects can be used to support the dosage adjustments during older patients' clinical trial design. [62]

3.2.2. Guidance on data extrapolation

Extrapolation can be defined as 'extending information and conclusions available from studies in one or more subgroups of the patient population (source population(s)), or in related conditions or with related medicinal products, in order to make inferences for another subgroup of the population (target population), or condition or product, thus reducing the amount of, or

general need for, additional evidence generation (types of studies, design modifications, number of patients required) needed to reach conclusions'. [63]

Quantitative methods should be used to support the data extrapolation, related to disease, drug pharmacology and clinical response to treatment, based on the effects' knowledge in the source population and the knowledge or assumptions about factors that can modify those effects in the target population. [63]

This tool could be used to overcome lack of clinical trial evidence for the safe and effective medicine use in a specific populational group, such as the geriatric patients, based on well-established knowledge in a different populational group.

EMA has issued a reflection paper on 'The use of extrapolation in the development of medicines for paediatrics'. This paper helps generating evidence to be used for the regulatory assessment of MA, clarifying different procedure steps to execute the extrapolation and situations where extrapolation cannot be applied. There are three steps: [63]

1. Extrapolation concept: identification of available data that allows establishment of a relationship data for the extrapolation execution; identification of knowledge gaps that need further investigation;
2. Extrapolation plan: definition of trials and tests for the identified gaps from the concept step, to produce evidence confirming the possibility of extrapolation, or identify the need to get some variables from the target population to allow extrapolation, or conclude that the gap will not allow the extrapolation;
3. Mitigation of uncertainty: continuation of the extrapolation plan in post-authorization phase to improve knowledge in specific uncertainty that will improve the labelling and the use in clinical practice.

No such guidance was executed for the geriatric population although it is stated that the principles in the existing pediatric paper can be extended to other areas.

3.2.3. Low-interventional clinical trial

Low-interventional clinical trial concept is introduced by Regulation (EU) Nº 536/2014 (see chapter 2.1) and it is a useful tool to generate evidence in the geriatric use of already marketed medicines that have not been appropriately studied in this populational group.

There are several factors that might contribute to a successfully enrollment of geriatric patients in this type of clinical trial, overcoming their current underrepresentation in the medicinal products development:

- The associated low risk of these trials because safety and efficacy has already been assessed in other populational groups during trials submitted in the application for MA;

- Possibility to include in the trial design the modelling and extrapolation techniques (specific geriatric patient guidance to be developed), supporting the evidence generation by the investigator's team and reducing the fears for unexpected adverse reactions;
- Increase of investigational team confidence for the inclusion of the elderly in the selection criteria;
- Increase of geriatric patient confidence for participation acceptance.

Low-interventional clinical trials should be encouraged by specific regulatory for geriatric-evidence based medicines development, still to be generated.

3.2.4. Real world evidence (RWE) and observational studies

Example of COVID-19 observational studies [64]

At 10th of May 2021, it was held a workshop to discuss the importance of global collaboration and information sharing in relation to real-world evidence obtained through observational studies, to facilitate regulatory decision-making on COVID-19 treatments and vaccines. This workshop was promoted by the International Coalition of Medicines Regulatory Authorities (ICMRA) and co-chaired by Health Canada and EMA. It was attended by participants representing 28 medicines regulatory authorities and experts from the World Health Organization (WHO).

ICMRA is a network of regulators that provides an effective platform to share valuable information on medicinal products and expertise. It facilitates successful collaboration on important studies to evaluate the effects and risks linked to the COVID-19 virus, its treatments and vaccines. This was the case with the management of rare thromboembolic events occurring following vaccination.

Not all represented countries regulators use RWE for regulatory guidance and decision making (example, Brazil, Saudi Arabia, Japan, USA on a case-by-case basis). Canada already used it before COVID-19, Denmark has a good registry of vaccinated individuals which allows close follow up, exchanging information with Sweden and Norway. Others, as African regulator, has additional concerns with necessity of healthcare providers training on collection of data and implementation of a standardized methodology.

EMA representative stressed that in COVID-19 randomized clinical trials studies for treatments and vaccines the excluded populational group has been pregnant women, who can most benefit from these observational studies.

The biggest consensual challenge mentioned was the capacity to data reliability assurance.

Extending the COVID-19 experience

Real-world evidence generated by high quality observational research is fundamental to complement the medicines benefit- risk understanding generated by the randomized clinical trials, when used in real world clinical practice dealing with concomitant therapies, comorbidities and populational groups underrepresented.

The ICMRA successful example given by the results with COVID19 can support and encourage the application of RWE from observational studies in other situations where lack of evidence challenges the safe and effective medicines use just as it happens with the current situation for the geriatric population.

Having already developed a worldwide medicines regulators organization and an information exchange platform, gained experience and still more to come with treatments and vaccines for COVID-19, steps are given for a global improvement of medicines specific for geriatric prevalent diseases (cardiovascular, cancer and neurodegenerative diseases) in a globally growing elderly population.

Adequate guidance for high quality observational research development is available in the site of European Network of Centers for Pharmacoepidemiology and Pharmacovigilance (ENCePP).

3.3. Ethics and the ability for consent

The clinical trial subject rights protection is, among others, regulated through the mandatory signature of the written informed consent which is the decision by the subject to voluntary participate in the study after receiving complete and understandable information on the purpose of the trial, the experimental drug and the use or not of placebo, procedures to be applied, reason for the subject inclusion, the potential benefits and risks. The volunteering for participating in a clinical trial is done under circumstances very well defined in the legislation, including an obligation from the clinical trial team to take special attention to vulnerable populations and incapable of giving consent, as well the subject right to withdraw the trial in any time without any negative counterpart (see chapter 2).

Under the expectation for a future increase in the participation of geriatric patients in clinical trials and the high prevalence of cognitive diseases in this group, it becomes urgent to assure the reliability of the informed consent signed by subjects with some degree of dementia or deciding on the necessity of having it signed by a legal representative.[65] A note should be done for the process by which this legal representative is designated because it might, also, pose additional risk for the subject.[65]

The decisional capacity is a process with four main components: capacity to understand the information and issues of the decision (comprehension); capacity to understand that the decision applies to the own person and to personalize the decision according to the intimate

values or beliefs (appreciation); capacity to evaluate different alternatives and their consequences (reasoning); capacity to communicate a decision (choice). [65] Additionally, emotion and social factors also contribute to the decision making. [65]

Patients with dementia become a vulnerable populational group due to the loss of decisional capacity. [65] There is an increased risk of making decisions against their own interest without realizing it (lack of awareness) and/or of being deprived of fundamental rights by others. [65] Additionally, the decisional capacity of patients with mild cognitive impairment may be excessively underestimated, although the many studies indicating that the decisional capacity can be affected in the early stages of cognitive decline. [65] Other geriatric prevalent pathologies that might have impact in decisional capacity are: decrease in sensory acuity, depression and stroke (especially in the case of aphasia). [65]

The determination of the decisional capacity can be done using several tools such as MacArthur Competence Assessment Tool for Clinical Research (MacCAT-CR) and University of California Brief Assessment of Capacity to Consent (UBACC). [65]

The MacCAT-CR tool is a structured interview with 21 questions divided into four subscales that allows the assessment of the four main dimensions of decision making process: understanding, appreciation, reasoning, and expressing a choice. [65] The questions are adjusted to the specific research context in which the patient is asked to participate. [65] There are some constraints in this test which are: there is no threshold or limit score that would directly discriminate patients able to decide from those who are not, it has some complexity (needs specific training) and the duration of the test is around 20 minutes. [65] These constraints can become a barrier for the inclusion of the patients in the study. [65] This tool was conceived to be an aid used by the assessor for the appreciation of decisional capacity and it is the most widespread and the most validated of all tools. [65]

The UBACC tool is a 10-item questionnaire and it is performed through a teach-back process of the protocol and the potential risks and benefits of participating in the clinical trial. [65] The total score is determined by the accuracy of answers depending on expected answers for each question. [65] It has some advantages: it is short, does not require specific training and can be adjusted specifically to the clinical trial proposed to the patient. [65] It has the disadvantage of not evaluating the capacity to express a choice. [65]

The lack of awareness (anosognosia) is correlated with disease severity but can affect patients at an early stage. [65] There are specific tests for anosognosia (such as the Anosognosia Questionnaire for Dementia) that should be used when evaluating patient's competence, as awareness and decisional capacity could be affected differently in patients with cognitive impairment. [65]

Considering the ethics and ability for consent circumstances related to geriatric patients' participation on clinical trials and the assessment tools here presented, it is suggested the inclusion of a mandatory decisional capacity determination step in the clinical trial protocol, to be executed before the signature of informed consent.

3.4. Geriatric patient-centric pharmaceutical product design

The patient-centric pharmaceutical product design consists in the identification of the target patient population specific needs to design products that will provide the best benefit-risk profile for that population. [66] It should be considered the patient's physiological, physical, psychological and social characteristics. [67]

EMA has adopted in 2020 a reflection paper on 'The pharmaceutical development of medicines for use in the older population' to encourage the pharmaceutical industry in the direction of a patient-centric approach to pharmaceutical development. [67]

The study of a medicinal product safety and efficacy in clinical trials is compromised by the quality of the data obtained. This data is highly dependent on the patient adherence to the therapeutic design, which can be defined as 'the route of administration, type of dosage form, formulation, strength/volume, dosing frequency, container closure system, measuring or administration device and the user instructions in the product information' [67]. The difficulty of adherence to a therapeutic design can also originate the drop out by the geriatric patient from the trial, contributing to the already existing deficit in the scientific therapeutic knowledge for the elderly.

In a concept where the clinical trial is designed to essay what is expected to find in real life, it becomes crucial having a pharmaceutical development since the beginning aligned with the specificities of the geriatric target population, to facilitate the therapeutic adherence and to have actual health gains in this growing population group.

When evaluating an application for approval of a medicinal product that will be used by geriatric patients, it should be a mandatory item to evaluate the adequacy of the pharmaceutical drug product design for this populational group

3.4.1. Impact of pharmaceutical design in the medicine acceptance

Most of geriatric patients are poly-medicated for different concomitant diseases with different chronic therapeutic regimens which might become a challenge to accomplish a correct medication and patient adherence.

There were identified several potential factors influencing the therapy adherence, related to: [68]

- Patient: morbidity/ co-morbidity, physiological capabilities, cognitive abilities, health beliefs, race/ gender, socio-economic status;

- Drug: therapy understanding, administration schedule and number of drugs, packaging, perceived effects and adverse effects;
- Environmental: relationship/ communication with the physician/ pharmacist; community support.

Considering aging-related dysfunctionalities some questions with impact on therapy adherence should be raised during the development of a pharmaceutical design for geriatric use:[68]

- Hand fine motoric difficulties/visual impairment: facility to release the medicine out of the package (example, blisters), management of small tablets, necessity to split a tablet for a correct dosage (availability of lower dosages), capacity to read medicine handling instructions;
- Hearing and visual impairment: oral and/or written therapy explanation is understandable, adequation of the product labels size letter (minimum of 12 size points), use of pictograms/ holograms, differentiation of medicines using colors in the package and dosage form;
- Cognitive loss: need of external support, mechanisms to remember the different regime for each medicine (examples: weekday printed in the blister, elaboration of a schedule associating daily activities with the necessary medication timings), patient meaningful treatment information (indication, benefits, treatment duration, adverse reactions) available in a simple language and structured as a list format;
- Swallowing difficulties: achievement of optimal tablet/ capsule size (not too small not too big), type of oral solid swallow ability (example, capsules are easier than coated tablets, coated tablets are easier than uncoated tablets), existence of alternative dosage forms for the conventional oral solid;
- Changed pharmacokinetics/pharmacodynamics: availability of an adequate dose, without dosage form manipulation;
- Concomitant diseases: existence fixed dose combinations for the patient condition, overcome the existence of drug- drug interactions.

In summary, when designing a medicinal product to be used by the elderly, the target product profile should include: easy to swallow, easy-to-handle, packaging easy-to- open, easy-to-dose, use of dosing devices, low risk of excipient overload, fixed combinations.[66]

3.4.2. Inappropriate use of medicines

The main route of medicinal products administration in the elderly is the oral solids pharmaceutical dosage form which become a problem in the frequent dysphagia situations. It became common the tablets crushing and capsules opening to use its content, very often mixing multiple medicines with food or a thickening agent.[69] This procedure originates loss of

drug during the crushing, loss of drug due to patient not taking all the food or refusing to eat due to bad taste (with implication on patient losing weight) and changed drug bioavailability with impact in the safety and efficacy. [69] There is an additional problem if this is done with enteric- coated drugs (risk of gastric damage and drug inactivation, with loss of efficacy) or slow release/ controlled release drugs (risk of overdosage and toxicity). [69] The alteration of solid dosage forms is considered a drug off-label use (legal implication), with the risk of administration without the consent of the subject (mixed in the food) and the risk of cross contamination if done in an environment of several patients being taken care. [69]

In the development of pharmaceutical drugs for clinical trials with geriatric subjects, alternatives to conventional tablets and capsules should be designed.

The interaction of the medicine with different types of food and drug-drug interactions should also be studied in the formulation alternatives, in order to be described in the patient information in case there is no alternative available.

3.4.3. Alternative technologies and administration routes

Here are presented some alternative methods of drug administration to be considered in clinical trials when developing medicines that will be used by geriatric patients.

Orodispersible dosage forms

Being a practical and familiar route of administration, some improvements were developed to keep the solid oral medication possible for medicines use even in the presence of dysphagia.

The orodispersible dosage forms are systems that dissolve or disintegrate rapidly in the oral cavity upon contact with saliva, without the need for chewing or addition of water, enabling an easier swallowing process. [70] They exist in the form of tablets and films (thin strips), being the tablets the preferred form for geriatric patient because they are more easily handled. [66]

The orodispersible tablets are already used for conditions common in older population such as pain, depression, Parkinson's disease and Alzheimer's disease. [70]

Limitations that are under resolution are the balance between appropriate mechanical strength and fast disintegration time, incorporation of high doses or poorly soluble drugs, taste masking for unpleasant drugs and the ability to sustain release. [70] Coated dextrin microcapsules were developed to increase absorption and mask taste, β cyclodextrin inclusion complex were developed to increase solubility and speed up the disintegration time, drug-loaded chitosan nanoparticles coated with polymer layers were used to achieve sustained release. [70]

There is impediment to use in geriatric patient with xerostomia unless some water is used. [70]

Fixed dose combination

These have been designed to simplify and reduce the solid oral dosage regime, improving the medication adherence. [66] It presents with some challenges like the adjustment of dose, PK/PD of each active ingredient, finding a common formula for compatibility with the excipients, interaction between the active ingredients, the final size of the dosage form being too big for oral administration. [66] To overcome these issues it has been used several technologic approaches: co-encapsulation of different solid drugs into hard gelatin capsules, direct compression, dry granulation, spray drying, wet granulation, multilayer tablets, multi particulate formulation, oral fast dissolving dosage forms and 3D printing technology. [66]

The fixed dose combination has been successfully used on the treatment of several diseases that are common in geriatric patients such as cardiovascular diseases, respiratory diseases and type 2 diabetes. [66]

Pulmonary delivery

The use of the large and thin surface area of alveoli rich in blood vessels allows subsequent systemic absorption even of high molecular weight compounds, with additional benefit of the low enzymatic activity and avoidance of first-pass metabolism allowing higher bioavailability and rapid onset of action. [70] It is an option for oral low bioavailability drugs, allows reduction in the dosage, administration frequency and side effects. Its use can be extended from the conventional asthma and chronic obstructive pulmonary disease to inhaled antimicrobials, vaccines, anti-hypertensive, diabetes (insulin), Parkinson's disease drugs. [70]

A setback can be the misuse of the inhaler due to decreased in the fine motor skills, vision and cognitive function of geriatric patients, becoming particularly important the appropriate education and training. [70]

In geriatric patients the alveoli absorption capacity is reduced (due to rigidity and thickness of alveolar surface and vasculature) and the respiratory capacity decreased. [70] The participation in clinical trials by geriatric patients that are going to use this administration route is crucial to obtain correct safety and efficacy information (their) for this novel administration route in these medicine indications, which until now is scarce and no reference is made on how to extrapolate data obtained in younger ages. [70]

Nasal delivery

This administration route with its high vascularization and large surface area, allows rapid systemic absorption and avoids gastrointestinal and hepatic first-pass metabolism, allowing higher bioavailability and dose reduction. [70] It also allows delivery of drugs to the brain. [70]

Alzheimer's disease and allergic rhinitis are already being studied for the use of drug nasal delivery. [70]

The enzymatic activity, mucociliary clearance, hydrophilic molecules and larger biomolecules are disadvantages that need to be solved. [70] It is already under study the use of mucoadhesive systems, permeation enhancers, enzyme inhibitors and nanotechnology to overcome these issues. [70]

In the elderly, there are physiological changes with impact in the successful use of nasal delivery systems such as decreased air flow, dry nose, intranasal crusting and nasal obstruction. [70]

Transdermal delivery

The use of transdermal application of drugs designed for systemic absorption requires specific physicochemical properties such as low molecular weight, a certain degree of lipophilicity, low melting point and high potency. [70]

There are already developed transdermal medicinal products for some psychopharmaceuticals, Alzheimer's disease, Parkinson's disease, angina, pain and hormonal disturbs. [70] Despite the changes that occur in skin during aging process (lose elasticity, increase dryness, thinning epidermal, decreased dermal vascularity, gradual deterioration of epidermal immune response and changes in endocrine environment due to endogenous skin aging) the passive transdermal drug absorption has shown no significant differences between young and old subjects. [70]

The drugs that passively permeate across the skin are limited and currently it is being study active transdermal delivery systems with microneedles technology (drug coated microneedles, soluble microneedles with drug incorporated, empty microneedles for drug infusion through them, use of drug reservoir). [70]

The use of transdermal drug therapy has important advantages for the geriatric patient: non- invasive, simple self-administration, reduced dose frequency (well controlled delivery rate achieving a steady-state effect), reduction of adverse effects, increased bioavailability (no first pass metabolism or enzymatic degrading action). [70]

The main disadvantage is the potential skin sensitivity and local adverse reactions which is augmented by aging. [70]

3.5. Geriatric subjects' enrollment in COVID- 19 vaccines' clinical trials

As of June 2021, four vaccines are approved and marketed in European Union: Comirnaty, COVID-19 Vaccine Moderna, Vaxzevria (previously COVID-19 Vaccine AstraZeneca) and COVID-19 Vaccine Janssen.

Unlike the extensively described exclusion of geriatric subjects from clinical trials during medicines development with intended use by this populational group, all COVID-19 vaccines approved by EMA included subjects 65+ in their trials.

What was the key for this successful enrollment?

In May 2020, attention was called for the need to develop coordination mechanisms in the development of COVID-19 clinical trials.[71] Hundreds of small independent clinical trials were running worldwide, most of them with scientific sound without commercial sponsors, raising questions if useful results were delivered for the disease knowledge/treatment/prevention as well as ethical concerns by exposing human subjects in researches with high level of uncertainty.[71] Among others, it was stressed the need to join efforts in large studies, use of large platforms for trials information sharing, inclusion of representative population (including the elderly, pregnant woman and younger participants), implementation of a close interaction between researchers and drug regulators and international coordination between the regulators themselves.[71]

In June 2020, a ICMRA workshop took place, co-chaired by EMA and FDA with 28 medicines worldwide regulatory authorities and WHO participation with the aim to agree on key principles of trial design for the acceptance of phase 3 COVID-19 vaccine trials.

In alignment with the results from the international regulators workshop, EMA issued in November 2020 a document with considerations on COVID-19 vaccine approval.[72] As a clinical requirement for the vaccine MA it was clearly written the demonstration of the safety and efficacy in adults including individuals with pre-existing comorbidities and individuals aged above 65 years, in at least one well-designed large scale phase 3 trial.[72] It was also stated that close communication with vaccines developers has taken place to facilitate trials execution according to the regulatory requirements.[72]

The factors influencing geriatric subjects' enrollment in COVID-19 clinical trials were:

- Effective international regulators organization with fast decision- making and successful alignment in the definition of global requirements in clinical trials phase 3, where usually excluded populational groups were integrated (example, subjects 65+);
- Effective communication (timely, objective and supportive) between regulators and vaccines developers on the regulatory requirements for their products acceptance;
- Execution of large-scale clinical trials (as required) involving several countries and thousands of participants which increased the geriatric population availability;
- COVID-19 high lethality rate among the elderly, increasing the probability of enrollment acceptance by geriatric subjects;
- Pandemic scenario with millions of people requiring vaccines, raising profitable interests by pharmaceutical industries and assuring the compliance with the requirements to obtain regulators' MA.

The challenge arising from these achievements is the future ability from the several stakeholders (regulators, pharmaceutical industry, scientific bodies, subjects) to apply the same mechanisms under a non-pandemic environment, for the development of the non-communicable diseases' therapies prevalent in geriatric population.

In developed countries, this populational group is increasing and sharing the same health problems, which turns this global approach of great interest for the improvement of public health.

4. Marketing authorization in EU: pediatric gives a hand to geriatric

Since 2006, Regulation (EC) Nº 1901/2006 'Medicinal products for pediatric use' was adopted to facilitate the development and accessibility of medicinal products to be used in pediatric population.

The adoption of Regulation (EC) Nº 1901/2006 was driven by circumstances that are common to both pediatric and geriatric populations: [73]

- Lack of appropriate studies to assure the safety and efficacy use of medicinal product in the target population;
- Utilization in pediatric population of existing medicinal products without studies or authorization for such use, originating problems such as: inadequate dosage information, increased risk of adverse reactions (including death), ineffective treatment (under-dosage), unavailability of suitable formulations and route of administration, unavailability of therapeutic advances;
- Insufficient market interest to stimulate the adequate research, development and authorization procedures.

It was intended with Regulation (EC) Nº 1901/2006: [73]

- Ethical and high-quality research, through studies well controlled and monitored according to requirements for the protection of pediatric population;
- Improvement of available information for the medicines use in the several pediatric sub-groups;
- Caution to avoid execution of unnecessary pediatric clinical trials;
- Adequate management to avoid delay the authorization of medicinal products for other age population.

These goals were achieved by: [73]

- Establishment with the pharmaceutical industry of a system of obligations and rewards and incentives;
- Creation of a scientific committee (the Pediatric Committee) with expertise and competence in the development and assessment of all aspects of pediatric medicinal products, ruled by the same legislation as the already existing committees (Regulation (EC) Nº 726/2004);
- Creation of mandatory Pediatric Investigation Plan (PIP) or approval of a waiver/ deferral. PIP is the document used for the authorization of the medicinal product for pediatric use. It should:
    - Indicate the subgroups of pediatric population to which it applies;
    - Describe the methodology and timings for the study of those sub-groups;

    - Be able to demonstrate the quality, safety and efficacy of the medicinal product in this population.
- Pediatric Committee making scientific assessment and agreement on both PIP or waivers and deferrals;
- Integration the PIP into the development of medicinal products for adults, if existing potential use in pediatric population, with PIP early submission to authorities to have the necessary studies executed before the submission of MA applications;
- PIP submission or proof of waiver necessity in case of MA application, application for new indication, new pharmaceutical form, new route of administration.

This MA procedure implemented for pediatric population is suitable to be applied under the same terms to medicines with intended use by the geriatric population. The adoption of a Regulation 'Medicinal products for geriatric use' was suggested (binding in its entirety and directly applicable in all Member States), as well as the creation of a Geriatric Committee and implementation of the Geriatric Investigation Plan (GIP).

5. Conclusions

Demographic European profile shows that 28% of population will be 65+ years old and life expectancy at birth will reach 85 years by 2050 and worldwide there also a trend for an increase in geriatric population. However, healthspan is not accompanying this trend, with 16-20% average of late-life time with morbidity.

These data have led science into two ways of action. Geroscience is digging deep into the knowledge of aging mechanisms and age-related diseases trigger. The goal is to obtain an effective disease prevention, using this knowledge to obtain evidence- based methods to succeed in aging effects delay, improving healthspan and quality of life in the elderly and, at the same time, unburdening the health systems. On the other hand, it will continue to be crucial the development of medicinal products to deal with the diseases prevalent in the growing geriatric populational group.

The understanding of the geriatric specificities (physiological/psychological changes and disabilities, co-morbidities and concomitant therapies), is clearly demonstrating that a straightforward extrapolation of data from studies in younger adults cannot be done, proving to be unethical to continue developing medicines that will be used by this populational group without its direct participation in the studies before approval of the marketing authorization, or preventing the access to the latest treatments due to lack of data. It is therefore urgent to overcome the underrepresentation of the elderly in the clinical trials and to obtain an evidence-based benefit-risk balance during the development of elderly-directed medicines.

A close look into binding regulatory governing the human medicinal products in Europe, USA and Japan has shown that there is no instrument in the law adopted for the elderly, despite the guidance on how to proceed with geriatric clinical trials and, recent this year, on how to adjust formulations. In Europe, the current clinical trials Directive and new Regulation to be applied early next year have a general requirement for the representation of the medicinal product target population, added of necessity to define eligibility criteria (inclusion and exclusion) and this has been used to exclude the elderly, either directly by definition of age limits or indirectly by the exclusion of concomitant diseases common in this population.

The key stakeholders implicated in the geriatric medicinal products development are the pharmaceutical industry, regulators and geriatric patients.

Concerning the pharmaceutical industry, the experience with COVID-19 vaccines and pediatrics Regulation has shown the efficacy of two actions to succeed in having special population subjects defined in the clinical trials inclusion criteria and a real effort for enrollment and data collection:

- Existence of a strong incentive (market driven or regulatory);

- Making the marketing authorization approval subordinated to the benefit-risk evaluation evidence.

From the regulators, the experience with COVID-19 vaccines and pediatrics Regulation has delivered several ideas with impact on industry adjustment of clinical trials for geriatric subjects' participation:

- Close interaction between regulators and industry for clarification and assurance of compliance with the regulatory requirements and scientific support on time for execution. An ongoing interaction should be established through the several steps of protocol elaboration, clinical trial execution and final report acceptance for the accomplishment of recommendations from chapter 3.1.2;
- Close interaction through international regulators' organizations to have common agreed requirements and facilitating the execution of large-scale clinical trials, reaching statistically significant target population;
- Adoption of a Regulation for geriatric medicines, uniformizing procedures among Member States, with creation of a specific geriatric committee and mandatory geriatric investigation plan, using same assumptions for industry benefits and MA subordination as already applied in pediatric Regulation;
- Additional ethics protection as a vulnerable population, with use of tools for decisional capacity evaluation before informed consent signature (see chapter 3.3);
- Encouragement of adaptive clinical trials demographic requirements;
- Assurance that IRB/IEC and investigator's team composition integrates geriatric expertise;
- Assurance of medicine development according to a geriatric patient- centric approach;

For the geriatric patients, there are several factors that both industry and regulators must take into consideration to accomplish with their voluntary enrollment acceptance in the clinical trials, reduction of the dropout and promotion of therapy adherence for a reliable collection of data during the study and real health benefits after the medicinal product marketing:

- Development of a home-based clinical trial culture (see chapter 3.1.2);
- Recruitment by geriatric patient trusting medicine provider;
- Clear communication of the procedures and benefits from participation on the study;
- Development of formulations, dosage forms, packaging, labelling and administration routes that allow the most independency possible for the geriatric subject condition/ disabilities and eliminate medicine manipulation for a successful administration and correct dose availability;

If all these measures still allow to anticipate an underrepresentation of geriatric subjects in a certain medicinal product development, additional actions to be evaluated by both industry and regulators are proposed:

- Inclusion in the geriatric investigation plan a pos-marketing data collection (observational studies);
- Evaluation if low- interventional clinical trial procedures are adequate;
- Development of guidance for extrapolation use in the development of medicines for geriatrics;
- Use of PBPK modelling and simulation to support dose determination.

In conclusion, there is still a big room for regulatory improvement to assure industry engagement in a geriatric- medicines development evidenced- based, also taking patient-centric approach as the way to achieve the goals of therapy adherence and correct use of medicine for the best health benefit achievement.

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Annex 1- World Medical Association Declaration of Helsinki

October 1996 17.C

Original: English

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI [27]

Recommendations guiding physicians in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964

and amended by the
29th World Medical Assembly, Tokyo, Japan, October 1975
35th World Medical Assembly, Venice, Italy, October 1983
41st World Medical Assembly, Hong Kong, September 1989
and the
48th General Assembly, Somerset West, Republic of South Africa, October 1996

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the etiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research, a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8 In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental 3 17.C incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE
(Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS
(Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

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