Work package 2

“Biogerontology”

Report of the WhyWheAge workshops

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A European road map for molecular biogerontology

1. Executive summary

Europe faces the immense challenge of unprecedented increase in life expectancy, which will become more intensive into the 21st century. Although this state of affairs is the essentially positive outcome from multiple improvements in health care and socioeconomic circumstances, it nevertheless leads to major pressure on all member and associated states of the European Union in terms of increasing prevalence of age-related health problems. There is urgent need for more basic research on the underpinning science of biological ageing, in order that it shall be possible to minimise dependency and improve health and quality of life for the rapidly growing numbers of older people. Therefore the next step to reinforce the constructive results arising from previous FPs was to establish a road map for European research on the molecular aspects of healthy human ageing. The WhyWeAge support action facilitated a coordinated series of research workshops on eleven topics considered as most relevant to human ageing research by the current and past coordinators of European projects. Based on an assessment of the state of the art in molecular gerontology and breakthroughs in biotechnology, we aimed to identify the main priorities for the next 10 to 15 years through the engagement of an open and transparent mechanism supporting a coordinated series of 11 research workshops on identified topics that terminated in an all-encompassing integrative conference where the texts of final documents were negotiated.

The ultimate goals are

1) To inform the various stakeholders including the European Commission dealing with scientific research via the production of a policy document entitled ‘A road map for molecular gerontology research in Europe’

2) To inform industry and point out potential areas of commercial interest

3) To inform health care providers regarding current state of the art

4) To inform the public.

The 11 main researches developed were :

1. Biomarkers of ageing and longevity

Reliable assessment of the state of ageing of a living individual is currently not possible. A strategy to solve this problem is the identification of (an) age-related change(s) in body function or composition that could serve as a measure of “biological” age and predict the future onset of age-related diseases and/or residual lifetime more accurately than chronological age does. Such parameters are termed “biomarkers of ageing”.

2. Telomere biology, DNA damage, mitochondrial biology and senescence

Telomere shortening, nuclear and mitochondrial DNA damage and repair, mitochondrial dysfunction and cell senescence are basic mechanisms that synergistically contribute to ageing. Importantly, these processes are tightly interlinked by multiple signaling networks and response pathways. Recent research suggests these as potent candidate targets for interventions aimed at extending healthy lifespan in model organisms and, ultimately, humans.

3. Oxidative stress, protein damage and maintenance

Protein damage combined with an age-dependent decline in anti-oxidative capacity and protein quality control result in an imbalance of protein homeostasis, that has been clearly linked to ageing. Indeed, oxidized protein accumulation is a hallmark of cellular ageing while protein aggregate formation has been also implicated in the pathogenesis of several age-associated degenerative diseases.

4. Systems biology

Systems biology is necessary to make progress in understanding the underlying biology of ageing. The importance of individual tools and approaches is dependent on experimental design, biological objectives, and data, which means that systems biology expertise is needed at every step of ageing research, starting from experimental design and ending with result interpretation, documentation and dissemination of the results. Systems biology can also bring in methods from fields outside biology, such as reliability theory.

5. Immunosenescence and inflammation

Dysregulated or compromised immunity (“immunosenescence”) in older adults results in their decreased control of infectious disease, poorer response to vaccination, and increased signs of systemic inflammation contributing to age-related diseases like diabetes, dementia and heart attacks. These diseases represent the biggest public health burden in our ageing population. Overcoming immunosenescence is thus a topic of crucial importance.

6. Metabolism

Recent advances in biogerontology are increasingly pointing to the central importance of metabolism as a determinant of ageing and health. Studies of metabolism, how it changes with age, and how interventions can slow ageing and increase healthy lifespan have the potential both to provide a deeper understanding of the biology of ageing and to improve late life health. In particular, a combination of recommendations in terms of lifestyle and nutrition, with appropriate drugs can be a powerful means of promoting healthy ageing.

7. Nuclear receptors

Nuclear receptors (NRs) organise and modulate physiological responses. Their ligand-gated transcriptional control coordinates environmental and nutrient signals with metabolism, development, reproduction, homeostasis, and, consequently, ageing. NRs have a long evolutionary history, thereby contributing to their complexity of action. They are prime drug targets but increased knowledge is required of their specificities and cross-talk in different aspects of ageing.

8. Vascular Ageing

Ageing is the major risk factor for cardiovascular disease, nowadays the most important public health problem in the ageing European population. Vascular ageing is a phenomenon where biology, lifestyle and social factors converge to accelerate a pathophysiological process resulting in both subclinical and clinical events, ranging from cognitive decline to heart failure and stroke. Current therapeutic interventions do not tackle the manifestations of vascular disease specifically related to the underlying organismal ageing. To address this situation there is an urgent need to gain a detailed understanding of the complex interactions between the molecular, biochemical, morphological and functional aspects of vascular ageing.

9. Muscle weakness, sarcopenia and physical exercise

Preservation of muscle mass and strength are now recognised as prerequisites for healthy ageing. Amongst the elderly population, nearly half of males and over half of women aged 70-79 suffer from sarcopenia. Age-associated loss of neuromuscular integrity, reduced regenerative capacity, inflammation and anabolic resistance have a major impact on muscle function and physiological reserves. Adoption of an inactive lifestyle and poor nutrition further exacerbates sarcopenia, which is becoming increasingly compounded by intramuscular fat accumulation due to increased prevalence of obesity in the older population. This results in sarcopenic obesity leading to further loss of muscle mass and mobility and serious additional health risks, such as metabolic syndrome.

10. Skin age-related modifications, elastic tissues and skin biotechnology

Skin is the functional barrier between the outside and inside of the body, preventing water loss, protecting against UV, the entry of adverse environmental agents and microbes. Skin ageing results in impaired barrier function, accumulation of defects under the onslaught of daily insults and impaired repair. As a result of skin ageing, the incidence of non-melanoma skin cancer (NMSC) and chronic wounds increases, while the defence mechanisms and barrier functions decrease.

11. Clinical biogerontological research

Bridging the still substantial gap between clinical and experimental biogerontologists will facilitate the medical breakthroughs greatly needed for the European older population. In addition it is necessary to address the means to overcome obstacles regarding ethical regulations, patient characterization and follow-up, and biobanking activities.

2. Rationale for success

Europe faces an immense challenge due to the unprecedented increase in life expectancy. Although this state of affairs is the essentially positive outcome of multiple improvements in health care and socioeconomic circumstances, it nevertheless puts intense pressure on all Member States and Associated States of the European Union in terms of increasing prevalence of age-related health problems. There is an urgent need for more basic research for underpinning the science of biological ageing, in order to facilitate its translational applications to minimise dependency and to improve health and quality of life for the rapidly growing numbers of older people. A crucial step to benefit from the results and unique expertise accumulated in Biogerontology in the previous Framework Programmes and to put these results and expertise in perspective with the possible future developments of the biomedical field and (bio)technology, was to establish a road map for European research on the molecular aspects of healthy human ageing. In other words, in order to achieve maximum efficacy and obtain relevant results rapidly, it was considered desirable to better define the priorities of research on ageing.

Eleven topics considered as most relevant to research on human ageing by the current and past coordinators of European projects were selected for deliberation, namely, Biomarkers of ageing and longevity; Telomere biology, DNA damages, mitochondria biology and senescence; Oxidative stress, protein damage and protein maintenance; Systems biology; Immunosenescence and Inflammation; Metabolism; Nuclear receptors; Vascular ageing; Muscle weakness, sarcopenia and physical exercises; Skin age-related modifications, elastic tissues and skin biotechnology and Clinical Biogerontological studies. Research priorities in each field were intensively discussed during separate workshops held in different European countries, each bringing about twenty leading scientists at the table. Research priorities are first introduced in a short state of the art section in order to define properly the importance of the research to be developed in the future and its implications for European societies.

3. Research theme and results of the European consultation

3.1 Biomarkers of ageing and longevity

A. State of the art

Ageing has been defined as the time-dependent decline of functional capacity and stress resistance and is associated with an increased risk of morbidity and mortality. Ageing is a process that affects all tissues and organs of the body. The rate of ageing can differ significantly between members of the same animal species, including humans. In other words, “biological age” may differ from chronological age. It is estimated that genetic variation accounts for only about one third of the variability in the human lifespan. Therefore, environmental factors, including nutrition, must play a large role. But even under the most highly controlled experimental conditions, i.e. using model organisms and studying genetically identical individuals kept under identical environmental conditions, a significant degree of heterogeneity in lifespan can be observed, which has led to the conclusion that some stochastic elements (or some hitherto unknown factors not controlled for) also play a role.

The classical quantitative assessment of “ageing” relies on the analysis of mortality curves (Gompertz function) of populations. Therefore, at the level of the individual, reliable assessment of the state of ageing, i.e. the state of the above-mentioned functional decline and prediction of the risk of the onset of morbidity and the residual individual life expectancy, is not possible with this method. A strategy to solve this problem is the identification of (an) age-related change(s) in body function or composition that could serve as a measure of “biological” age and predict the future onset of age-related disease and/or residual life expectancy more accurately than chronological age does. Such parameters are termed “biomarkers of ageing”. At present, knowledge of biomarkers of ageing and longevity is scarce on all levels, be it in humans, model organisms or cells. For instance, knowledge of biomarkers of potential cellular senescence is available, but the currently best accepted marker, i.e. the colocalization of telomeres with DNA damage foci, is a technically demanding method and cannot be applied for routine use. Therefore there is a huge demand for further research.

A biomarker can be predictive of the years of healthy survival but can also be indicative of an already initiated but not yet clinically manifest disease. Therefore, the quest for biomarkers is always dependent and closely linked to the question asked. The EU FP7 Large-Scale Integrating Project MARK-AGE, for example, is one EU-funded project that represents an important and encouraging step in this direction. It was launched in 2008 and it aims at establishing a set of robust biomarkers of ageing in a European cohort of 3,700 men and women aged 35-74 years. Such an ensemble of several biomarkers with appropriate weighting should enable a better description of biological age than any single biomarker in isolation. It should be mentioned that this project in part draws on the existing cohort already recruited within the FP6 Integrated Project Genetics of Healthy Ageing (GeHA). The major limitation of the

ongoing MARK-AGE project is the lack of a significant longitudinal component due to restriction of project duration and finances. A longitudinal follow-up of the study subjects is, however, important for validation of the predictive power of the candidate biomarkers. Another important limitation of MARK-AGE is in the range of candidate biomarkers that could be studied, due to restriction of finances and type and quantity of biological samples that can be obtained in that project. Another example of EU-funded work that includes population studies on the ageing process is the FP6 Network of Excellence LifeSpan, which addresses the mechanisms of ageing especially in relation to development.

Research on biomarkers of biological ageing is a field of ever increasing importance, as their availability is a prerequisite for monitoring any interventional strategies including preventive measures against age-related diseases.

B. Recommendations

B.1 The biology of biomarkers and translational studies

In the search of biomarkers, we can categorize the research to several levels, i.e. human system (at the level of a whole organism but also at the cellular level) and non-human/organismal models. Studies on the cellular level will clarify the basic mechanisms that underlie biological ageing. Regardless of the system used, the **elucidation of the biological mechanisms** that drive ageing is a prerequisite in order to select the most powerful and specific biomarkers. The identification of **biomarkers of general biological ageing** is considered the holy grail of the field. Their measurement should be non-invasive or minimally invasive, fast, highly predictive, cheap, reproducible and safe.

Human models

Longitudinal studies of human ageing

A step in the right direction has been taken with the MARK-AGE project, for example (see above the state of the art). This kind of studies should aim at the identification of **organ and tissue specific biomarkers**. The **stratification of the examined population** is necessary and it should be done in different **age classes** and their distinct particularities i.e. young people, middle-aged people, older people and extremely old people, according to **gender, health or frailty status** of the subjects as well as **genetic profiles** of the individuals**.**

A clear distinction between healthy people being at risk of developing a certain condition and patients should be made. Furthermore, the identification of **biomarkers for individuals** (e.g., in order to decide the treatment regime of a certain geriatric patient) but also for **subgroups** is desirable, as the latter will narrow down multiple treatment choices.

Apart from the biological biomarkers, **social markers** should not be excluded from the field despite the fact that they could dilute out the objectiveness of a marker, given that studies have shown for example that perceived health status correlates with survival. Finally, the identification of **combinatorial/multifactorial biomarkers,** including genetics for stratification, might prove predictive, specific and selective.

Markers at the cellular level

Although the ultimate goal is the identification of biomarkers at the human organismal level, markers at **the cellular level** are an integral part of this, given that cellular ageing definitely influences the rate of functional decline of the body.

The **mechanistic relevance** of each biomarker has to be tested and characterized *in vitro* and *in vivo*. When the model of cellular senescence *in vitro* is applied, further attention should be given to the cell culture conditions, to the generalisation of the results to the whole organism, and to the distinction between specific senescence biomarkers and cancer biomarkers.

Finally, at the cellular level, although one of the well-characterised models for studying ageing is cellular senescence *in vitro*, reliable and easily accessible markers for **cellular senescence *in vivo*** are still lacking. This is of utmost importance since it is becoming increasingly clear that senescent cells *in vivo* exert detrimental influences on the tissue microenvironment.

Non-human models

The model systems proposed include mice, fishes, worms (*C. elegans*), flies (*D. melanogaster*) and yeast (*S. cerevisiae*) may provide a number of advantages. For example, **faster and easier longitudinal studies** with frequent sampling are possible in such organisms. The **combination of biomarkers** at the level of the whole organism is more applicable in the non-human model systems, and more analytical and statistical tools have been developed up to now (i.e. there are several algorithms that analyse multifactorial markers in mice and may even predict the death of the organism). There are multiple strains that can help experiments in various ways (e.g. transgenic mice).

Especially regarding mice there are strains that undergo very successful ageing simulating the human **centenarians**. A more recent advance in mouse experimentation is **humanised mice**.

Regarding lower organisms like *C. elegans* and *D. melanogaster*, there are several features that turn these organisms into powerful tools. For example, **functional genomic screens can be completed in only a few weeks time**. Moreover, **genetic manipulation is faster and easier** in these lower

eukaryotes with no ethical restraints, in contrast to mice. Although it appears that model organisms are really powerful and necessary, and should not be excluded from the biomarkers field, one should keep in mind that **any results from model systems ultimately require validation in humans**, to define what is really relevant to human ageing itself. Therefore, model organisms are most powerful in **the identification of underlying mechanisms** that will eventually reveal causal and potent biomarkers.
In brief, an axis of prime importance is to combine research in the human system with research on model systems in order to identify valid biomarkers with predictive potential. Only then preventive and regenerative therapies can be followed up properly during and shortly after treatment and not only in a long-term retrospective way.

B.2 Research infrastructure

The following components of research infrastructure should receive special attention:

- Longitudinal studies

One positive example of creating critical mass and infrastructure in Europe in the field of biomarker research is MARK-AGE. It is strongly recommended that such projects be continued as a **longitudinal studies**, which should further be connected to other studies including **interdisciplinary areas of biogerontology** (molecular biology, biochemistry, physiology, clinical biology and clinical studies etc).

- Central database

Existing and emerging databases across Europe should be identified and connected to create a comprehensive and well-curated **central database** guaranteeing fast and easy overview on the current knowledge in regard to biomarkers. This central database should also contain links to existing biobanks, like the one that has been created in UK, with large-scale sampling and an executive board deciding on the use of samples, so that emerging biomarkers can be rapidly tested in retrospective samples.

- Towards establishment of a European Biobank

It would desirable from a scientific point of view to create a European Biobank that provides blood products and tissues from human donors accompanies by respective background information. In view of the ethical issues and especially in view of the very different legislations, traditions and cultures across European member states, however, this appears to be not a trivial task. Therefore it is suggested to organize a workshop bringing together experts in medical ethics and law from a wide array of Member States, in order to review and discuss the existing differences and to explore possibilities for better harmonization, if possible. The results of such a workshop will tell if it is realistic to go for the establishment of a European Biobank.

3.2 Telomere biology, DNA damage, Mitochondria biology and senescence.

A. State of the art

Telomeres, the extremities of chromosomes, shorten with cell division and with ageing. If not fully counteracted by telomerase (an enzyme which repairs DNA), this eventually leads to chromosome 'uncapping', which induces a DNA damage response and triggers cell senescence. Even though telomere maintenance ensures cell immortality, intervention on telomeres can be associated with potential tumour risk, and therefore they might not be ideal targets for treatments. Their potential as biomarkers for ageing is under examination.

DNA damage occurs constantly as a consequence of normal cell metabolism. Elaborate repair systems have evolved for various types of damage, because persistent DNA damage can induce many disturbances in the cell. These repair pathways are of crucial importance for keeping levels of persistent DNA damage low, thus controlling the rate of ageing.

Mitochondria are the centre of major metabolic pathways and thus tightly interconnected with systemic regulation of nutrient utilization and cell growth, processes that determine the rate of ageing and are so far the best known targets for life-extending interventions. Mitochondrial DNA accumulates lesions with ageing in many organs, but the importance of low levels of mtDNA (mitochondria DNA) lesions for ageing is not sufficiently clear. Dysfunctional mitochondria are a main source of reactive oxygen species (ROS), which damage biomolecules in the cell.

Cell senescence was first observed as the irreversible and reproducible loss of replicative capacity in cultured human cells. Senescent cells interact differently with their environment because of profound changes in their gene expression pattern. Emerging evidence points to a significant role of cell senescence for ageing of organisms.

Telomere shortening, nuclear and mitochondrial DNA damage and repair mitochondrial dysfunction and cell senescence are basic mechanisms, which synergistically contribute to ageing. Importantly, these processes are tightly interlinked by multiple signalling networks and response pathways. Mitochondrial ROS production is an important cause of oxidative damage to nuclear DNA, including telomeres, and telomere shortening. Conversely, mitochondrial dysfunction and ROS production are also consequences of cell senescence. However, the connection between mitochondrial failure, specifically mtDNA mutations, and ageing appears not to be mediated primarily via oxidative damage.

B. Recommendations

B.1 The basic biology of ageing and translational studies

We see the following as the most important and relevant research questions for the next 10 – 15 years:

Telomeres

- Are telomeres reservoirs/sinks for DNA damage, which is not repaired?
- Are telomere-independent functions of telomerase important for ageing?

(Nuclear) DNA damage and DNA damage response

- Investigate the **whole spectrum** of endogenous DNA damage in ageing.
- We lack evidence for DNA damage accumulation *in vivo* in different tissues.
- Investigate the molecular mechanisms of the DNA damage response (DDR).

Mitochondria

- Relevance of mitochondrial parameters/functions other than ROS in the ageing process.
- The connection and cross-talk between mitochondria and the nucleus.
- Pharmacological strategies to improve functional efficacy of mitochondria.
- Mitochondrial DNA damage and repair.

Oxidative stress

- Develop decisive experimental approaches to test the impact of ROS in ageing.
- Find approaches to distinguish between different types of ROS and their effects *in vivo*.
- Develop more specific interventions that allow targeting of ROS-mediated damage but leave ROS-dependent signalling intact.

Cell senescence

- Difference and similarity between senescence and cancer.
- Accumulate a sufficient level of descriptive data on frequency and persistence of senescent cells in different tissues/organs/species with ageing.
- Examine the signalling pathway networks that induce/stabilize/maintain senescence.
- Would selective removal of senescent cells slow down ageing?

B.2 Infrastructure

At the level of **infrastructure**, **long-term funding** is necessary for **biobanking** and for large **longitudinal studies.** The issue of a central European Ageing Biobank was discussed, but there are big ethical and proprietary issues. Increased, long-term EU support for national/local biobanking (with the responsibility to make resources available over time) appears more feasible and more useful. Furthermore the long-term use of databases, biobanks generated in the context of EU should be co-ordinated and supported financially in order to maintain such precious resources and enable their widest possible use.

There would be large merit in setting up a **European Institute/College on Ageing,** modelled to a certain extent on the NIA. A discussion and decision-finding process should be instigated to decide on its principal structure (virtual or real) and its core aims and powers. Some of the relevant issues to be addressed are the following:

- multidisciplinarity
- provision of infrastructure (laboratories, animal cohorts)
- earmarked research funds / intramural programmes
- collaborative projects
- training / workshops
- sustained funding for long-term / longitudinal studies
- coordination of national projects
- possible involvement of Big Pharma

3.3 Oxidative stress, protein damage and protein maintenance

A. State of the art

The “Free radical theory of ageing” proposed 50 years ago underwent several revisions but its basic concept that the molecular damage by free radicals and other oxidants contributes to the ageing process remains essentially unchanged. However, since not only free radicals can damage biomolecules, a “Molecular damage theory of ageing” is preferred nowadays.

Proteins are constantly attacked and damaged by exogenous and endogenous agents. Protein homeostasis is the ability for the cell to maintain its protein content. Protein damage combined with an age-dependent decline in anti-oxidative capacity and protein quality control result in an imbalance of protein homeostasis, that has been clearly linked to ageing and age-related diseases. Maintaining a proper protein homeostasis turns out to be essential to insure healthy ageing. It has also become clear that free radicals do not merely damage macromolecules but also act as important mediators of normal physiological functions so oxidative protein modification may play also a role in cellular signalling.

Protein synthesis is an important determinant of ageing. In diverse organisms, from yeast to humans, ageing is associated with extensive changes in both general and specific protein synthesis. Protein damage plays a crucial role in normal ageing and in the pathogenesis of various degenerative diseases while failure of protein maintenance has been implicated in the age-related accumulation of damaged protein. Protein maintenance (protein quality control) implies sensing and eliminating of damaged proteins through repair and degradation combined with de novo protein synthesis. Inefficient degradation results in accumulation of protein aggregates, which is associated with ageing and age-related degenerative diseases. All interventions known to delay ageing reduce protein aggregation, suggesting an intrinsic link between protein aggregation and ageing.

B. Recommendations

B.1 Protein homeostasis in ageing

The “Free radical theory of ageing” was proposed 50 years ago. Despite multiple revisions and improvements, the basic concept that reactive molecules are damaging cellular components during the lifetime of an organism remains essentially unchanged. It became clear that one of the most important targets of such damage are proteins.

Understanding protein damage. Protein damage is a universal process but some proteins are more prone to damage and some types of damage are more detrimental than others. Tremendous work is still required to understand the detailed process of protein modification. Weighing the physiological consequences of particular modifications is a critical goal for future research in the field.

Cellular response to protein damage. Cellular reaction to protein damage needs to be further investigated. Indeed, only few damaged proteins are repaired and most damaged proteins are rather degraded. More basic knowledge about the regulation of these degradation pathways, their cross-talks and their interactions with other maintenance pathways is required.

Sensing and signalling protein damage. Responding to protein damage requires that cells can efficiently assess this damage. A wide array of consequences of protein damage should be analysed starting from the loss or switch of function of the damaged protein to the protein damage sensing machinery and the interplay of protein damage with cellular signalling. The age-related changes in these pathways deserve further attention while the impact of protein damage during development on late-life events and longevity needs to be addressed.

Role of protein aggregation. Beside and together with modification, alterations in intracellular protein homeostasis result in protein aggregation that has been associated with ageing and age-related (neuro-)degenerative diseases. Significantly, all interventions known to delay ageing also reduce protein aggregation, suggesting an intrinsic link between protein aggregation and the ageing process. The mechanisms underlying protein aggregates formation and its link with protein damage need to be further investigated. While some reports claim a protective role of aggregates, other have reported toxic effect of protein aggregates. It is therefore likely that more needs to be understood on the nature, the form and the reactivity of different aggregates.

The issue of protein synthesis. Ageing is associated with extensive changes in both general and specific protein synthesis which are not simply a corollary of the ageing process but, rather, they have a causative role in senescent decline. It seems that protein synthesis is a so far underestimated determinant of ageing. Deciphering the molecular mechanisms that bring about the changes in protein synthesis during ageing is an important issue.

B.2 Future design of interventional strategies

The finding of compounds and strategies to maintain or stimulate protein repair and degradation mechanisms, as well as stimulation of protein synthesis might be an effective strategy to prevent age- or disease-related failure of protein homeostasis. Furthermore, the removal of so-far undegradable aggregates should be also addressed pharmacologically and by means of biotechnology and might be also of future economic importance. In summary, strategies should be developed not only to avoid protein modification and aggregation but also to better cope with them.

B.3 Methodological developments and models required

Broad approaches that include high standards proteomics, metabolomics and systems biology are critically needed to address the complexity of damaged protein networks and the diversity of damaged protein targets in both basal and stressful conditions.

3.4 Systems biology

A. State of the art

The ageing process is highly complex and a fuller understanding will only be gained by applying an approach that takes into account multiple interactions at multiple biological levels operating over a wide range of time scales, ranging from molecular interactions taking less than a second up to life-course processes that develop over decades in humans. The task is daunting but much effort is being dedicated to developing such an approach within the emerging field of systems biology. It is essential that full use is made of developments as they arise but it is also essential that researchers in ageing are fully engaged in steering the specific developments that are necessary for ageing.

Systems biology and ageing

A central problem is to understand the role of single genes and gene products, molecular functions and cellular processes that contribute to ageing. These requirements map directly onto a useful formalisation defined within the Gene Ontology (GO) standard. For example, if the expression of a gene of interest in ageing is artificially prevented in an experiment, then software that employs GO can be used to investigate processes and functions that are affected. Technologies for data generation, analysis and storage are essential in ageing research as in many other areas of biology.

Another area of development in systems biology is the established iterative cycle of focused experimentation and dynamic modelling that is used for detailed investigation of particular hypotheses. Such as approach has been used successful to study telomere shortening and protein aggregation. The use of standards for representation of biological models has enabled more ready exchange and development of models between different groups.

Ageing and systems biology: specific insights and specific requirements

One way to gain an understanding of a system is to ask how and why it fails and ageing reveals the full repertoire of system failure. For example, robustness is a key property of a system and systems biology is ideally suited to its study. Ageing has a particular identity in that it is associated with a breakdown in robustness. Ageing is due to the accumulation of unrepaired damage and the study of progressive, irreversible alterations in systems is a distinctive feature. Stochasticity is also a key feature of ageing and particularly challenging to study.

B. Recommendations

Science

1) Top-down approaches in the study of individual differences in ageing

There are large differences in ageing rate between individuals. Genetics, environment and intrinsic chance are each known to play a role. Genetic factors associated with longevity are well described in model organisms and there is some correspondence with SNPs (Single Nucleotide Polymorphisms, small genetic variations that could occur in the DNA sequence of a person) that have been identified in humans. The function of these genes in actually affecting the rate of ageing is not well understood. Top-down systems biology is based on a hypothesis-free approach that offers a means to go beyond correlation and identify pathways or processes that are altered. The outcomes are hypotheses that can then be tested with focused experiments.

2) Understanding lifespan extension with dietary restriction requires a multi-level physiological systems approach

After over 70 years of study we still do not understood how dietary restriction extends lifespan in many model organisms and whether or not we can expect the same in humans. It is proving difficult to determine the underlying mechanisms as most known mechanisms of ageing are affected but the most robust observation is of metabolic reprogramming. We recommend development of integrated systems models that aims to characterise the effect of nutrition over a range of food levels.

3) Furthering studies in physiological systems biology

Physiological systems biology is not as developed as systems biology at the cellular level but it is required to understand how molecular mechanisms of ageing impact at the level of the whole organism. Two approaches that would provide valuable insight are: (i) the Physiome project, which is one of the only large physiological systems biology programs; we recommend adding an ageing dimension to each tissue/organ system that is currently being developed; (ii) variations in the function of the somatotrophic and hypothalamus-pituitary-gonad axis, both closely linked to ageing.

4) Clarifying a role for oxidative stress in ageing would be helped with a bottom-up systems biology approach

There is a growing body of data that does not align well with the hypothesis that molecular damage due to oxidative stress is a major cause of ageing. There is however much evidence in support. Explanations for the discrepancy include homeostasis and compensation in the antioxidant system and the important role that reactive oxygen species (ROS) serve in cell signalling. We recommend a bottom-up systems

biology approach that aims to study both the protective role of the antioxidant system and the role of ROS.

5) Cellular processes

Systems biology has been used to study cellular processes such as apoptosis and senescence but much remains to be done. We recommend studies that are able to initiate the process and then to generate comprehensive time course datasets on individual cells. The experimental program would be supported and guided by computational modelling. The models would be used to predict behaviour following perturbation and appropriate experiments will then be performed.

Infrastructure

Systems biology depends on technology. Up-to-date and reliable computing facilities are essential. Computing hardware must be maintained and software must be constantly updated. Applications that are web-service enabled are proving to offer major advantages as they enable researchers to design their own pipelines relatively simply. We recommend establishing a centre, which may be in more than one physical location, which has a broad computational biology dedicated to the ageing research community. The exact roles required would need to be established but would include an advisory and training role. This would act as a computing resource to include the difficult task of taking on research level software that proves to be valuable, bringing it up to industry standard and keeping it well maintained.

3.5 Immunosenescence and Inflammation

A. State of the art

Dysregulated immunity contributes to the four major causes of morbidity and mortality in older adults (respiratory disease, cardiovascular, stroke, cancer). Understanding ageing of the immune system will allow rational interventions to extend healthspan and reduce health care costs.

The immune system evolved to maintain homeostasis between host tissues and the internal and external microbiological environment to protect the integrity of the host. In all organisms, soluble factors are produced which protect against bacterial, viral and fungal invasion. In addition, in most animals, specialized cells dedicated to rapid recognition and elimination of pathogens developed; these cells use specific cell surface receptors recognizing molecular entities shared by invaders but absent from the host (innate immunity). In vertebrates, another class of cells (lymphocytes) emerged which recognize microorganisms by means of unique receptors formed by recombination of highly diverse genetic modules to generate a very large repertoire of different antigen-recognizing molecules. On contact with their targets, these cells undergo clonal expansion and differentiation into effector cells and memory cells; after elimination of the invader, a fraction of memory cells is retained to facilitate a more rapid specific response on rechallenge with the same pathogen. Hence this arm of immunity is designated “adaptive”.

It is widely believed that dysregulated or compromised immunity (“immunosenescence”) is causally related to the decreased control of infectious disease by the elderly, to their poorer response to vaccination, and to the increased signs of systemic inflammation often seen in older adults (“inflammaging”). The latter is implicated in a plethora of age-associated diseases including cardiovascular disease, Alzheimer´s Disease, type II diabetes etc. It may also play a role in carcinogenesis. Immunosenescence may become clinically important in different people at different ages, according to genetics and many other factors, including lifestyle, socio-economic status and the pathogen load to which individuals are exposed throughout life. Immune parameters predicting mortality have been sought for many decades, and establishing a robust set of biomarkers of immune ageing applicable to diverse human populations would pave the way for monitoring interventions aimed at restoring appropriate immunity in the elderly, as well as proving causation. Although many studies report associations between certain age-influenced immune parameters and mortality, most have been based on cross-sectional measurements on different young and old populations. These cannot compare like-with-like or establish causality. However, circumstantial evidence from a very small number of longitudinal studies, and mostly limited to very elderly humans, have begun to reveal “immune signatures” or biomarkers of immune ageing consisting of clusters of parameters increasingly recognized as an “immune risk profile”, or IRP. Establishing whether the IRP is a general characteristic of human

populations and understanding the mechanisms responsible for its emergence would allow design and validation of interventions to reverse the effects of immunosenescence.

B. Recommendations

B.1 Identification of a number of areas where there is an urgent need for further study

B.1.1 Improve understanding of the basis of reduced vaccination responses in older adults

The single most effective measure in preventive medicine is vaccination. However, it is well established that older adults may have a profoundly compromised response to vaccination, the basis of which is poorly understood. There is a real need to target vaccinology research towards improving these responses in older adults. In order to achieve this we need a better understanding of the cellular and molecular factors responsible for reduced vaccination efficacy with age.

B.1.2 Define the clinically-relevant components of immunosenescence that are important for maintaining immune health in old age

Beyond the obvious consequences for increased risk of infection, dysregulated immunity has a broad influence on health and affects many organ systems. Thus, there is a need to carry out research to investigate immune signatures associated with decreased efficacy of preventive strategies such as vaccination, and increased risks of age-related diseases. Such data would allow prediction and intervention to ameliorate these widespread and clinically significant diseases.

B.1.3 Determine the factors contributing to immunosenescence

It is now clear that a variety of genetic and environmental factors impact upon health in old age, including effects on immunity. However, the relative contribution of these factors to immunosenescence needs to be established. These variables include for example nutrition, genetic background, or socioeconomic status. This will provide the solid evidence base required to develop effective personalized interventions, both lifestyle and pharmacologic, to extend health span.

B.1.4 Integrating assessments of immune signatures into ongoing and planned longitudinal studies

Commonly, cross-sectional and longitudinal studies neglect acquisition of data on immunity. Because dysregulated immunity exerts systemic effects on many organ systems, and contributes to pathology, it is vital to include the assessment of immune parameters in such studies.

B.1.5 Develop and validate improved in vitro and animal models to better understand human immunosenescence

Current animal models are predominantly in rodents, the immune systems of which may not accurately reflect the human. Better models could include non-human primates, mini-pigs etc as well as the use of *in vivo* imaging of immune processes in appropriate models.

B.1.6 Assessment of existing interventions for their effects on immunosenescence in humans must be undertaken in a systematic manner

Interventions such as improved vaccination regimens, prophylactic vaccinations, nutritional interventions, use of existing drugs, physical activity could be rapidly implemented if proven effective and therefore should be promoted in the near future.

B.1.7 Development of cost-effective and practical diagnostic and monitoring tools for immunosenescence – with a view to facilitating inclusion of the measurement of immunological parameters in longitudinal studies, as well as validation of interventions.

B.2 Infrastructures

It is essential to accurately identify which of the multitude of alterations to immune parameters are causally-related to a person's clinically unfavourable health status, in order to determine the mechanisms of immune ageing. This is an enormous challenge, as it requires longitudinal studies in a very long-lived species. Small scale studies in very elderly humans have begun to reveal "immune signatures" or biomarkers of immune ageing consisting of clusters of parameters increasingly recognized as an "immune risk profile", or IRP, associated with earlier mortality in follow-up. Establishing whether IRP is a general characteristic of human populations and understanding the mechanisms responsible for its emergence would allow design and validation of interventions to reverse the effects of immunosenescence. This requires a concerted Europe-wide effort with long-term commitment of resources to enable exploitation of ongoing studies and to establish well-characterized cohorts that will include biobanking of appropriate specimens (including viable blood cells and tissue samples) and collation of health and lifestyle data. Such longitudinal studies will provide a vital resource for integrating multiple parameters correlating with morbidity and mortality at the level of the individual and provide an essential tool for multidisciplinary studies on human ageing.

3.6 Metabolism

A. State of the art

The term "metabolism" encompasses the processes by which organisms produce, maintain and degrade their material constituents and by which energy is produced. Advances in biogerontology increasingly point to metabolism as a determinant of ageing and health. Much insight has come from two approaches. (1) Studies of age-slowing interventions in animal models, including single gene mutations

and dietary restriction (DR), the controlled reduction of dietary intake. (2) Longitudinal studies of the biological changes accompanying the ageing process. Such studies show that factors such as diet and exercise can produce changes in metabolism that profoundly affect ageing and health. They demonstrate that appropriate interventions in metabolic processes can promote healthy ageing and reduce late-life pathology. Moreover, analysis of the pathways by which metabolic processes impact ageing can lead us to an understanding of the biology of ageing itself, one of the most long cherished goals in the search for human knowledge.

A powerful approach to the biology of ageing is to find **interventions** that alter its rate in animals and then study the mechanisms involved. Interventions that slow ageing (e.g. DR, single gene mutations and drugs) show that ageing is not a fixed and immutable process; in fact, it is relatively easy to manipulate in the laboratory. Such interventions may lead to decelerated ageing, which produce a reduction of incidence of the full spectrum of ageing related pathologies, and an increase in healthy lifespan. If successfully applied to humans, principles derived from these studies could yield great gains in terms of quality of life and health for older people in the future.

In **longitudinal studies**, many, central metabolic pathways show profound changes with age that appear to contribute to organismal ageing. Again, diet plays a key role. Studies of metabolism, how it changes with age, and how interventions can slow ageing and increase healthy lifespan can provide a deeper understanding of the biology of ageing, and improve late-life health. In particular, we foresee a combination of recommendations in terms of lifestyle and nutrition, and drugs with a powerful capacity to promote healthy ageing as a long term output of this research. This would allow people to live longer, healthier and more productive lives, and greatly reduce the individual, social and financial burden of late-life morbidity.

B. Recommendations

B.1 Future research needs to understand how metabolism and metabolic homeostasis are affected by diet, age and other factors. This covers many questions including:

a) What are nutrient sensing pathways that control ageing?

Effects of diet on ageing seem to be exerted, at least in part, via nutrient-sensing pathways. What are the signaling pathways involved? How do they talk to each other? Where are they operating and how do different sites of action/effect communicate with each other? Do all of DR's effects via signaling or are some due to dietary imbalance, or other factors?

b) What is the topology of gene regulatory networks linked to nutrient sensing pathways and what are the age related changes in chromatin structure?

Downstream of pathways that regulate ageing are complex gene regulatory networks. Many different transcription factors (TFs, proteins controlling gene expression) are involved in these networks. Of particular interest are TFs that function throughout development and ageing as molecular integrators of complex physiological regulations. The future focus should be on the transcriptional networks that assure metabolic homeostasis. The central role of key nutrient sensor nuclear receptors (LXR, PPAR) in these networks argues for focus on their effects.

c) Where anatomically do effects of metabolism exert their effects on ageing?

Do some organs play a greater role than others in the central regulation of ageing? Studies of tractable model organisms could help to better establish where lifespan regulatory events are occurring. A further critical question is how metabolism and ageing is coordinated between tissues, and the signals involved.

d) How does the brain control metabolism and ageing?

The brain and the body's metabolic/endocrine processes are bi-directionally linked. Dysregulation of homeostatic mechanisms during ageing is associated with increased morbidity and impairments in brain function. Studies of long-lived mouse mutants show the importance of brain regulation of ageing. Age changes in brain metabolism are poorly understood. Factors influencing peripheral metabolism and longevity often have good or bad effects on brain ageing depending of degree and/or context. Different brain areas and cell types need to be studied with regard to brain metabolism and ageing (for which recent technological advances in brain imaging will be crucial), and their function understood in the context of whole organism. This is a priority area for future research, given the growing evidence that metabolic syndrome (including impaired glucose tolerance, obesity, hypertension, hypertriglyceridemia, and reduced HDL cholesterol) may be important in the development of age-related cognitive decline, mild cognitive impairment, vascular dementia and Alzheimer's disease.

Interesting topics within this area include whether age-related neuronal and cognitive changes in humans implicate any of the master regulators of metabolism and ageing/ lifespan identified in model organisms. The links between metabolic signaling, stress resistance and formation of protein turnover and aggregate formation during ageing need to be addressed.

e) Adipose tissue, fat metabolism and ageing.

Recent studies have increasingly shown adipose tissue and fat metabolism to be central to ageing as well as a major regulator of metabolism. How fat metabolism influences lifespan is unclear, and this is new and exciting. Adipose tissue has many interesting effects on health that intersect with other elements of this road map.

f) What are the downstream biochemical processes that directly affect ageing?

Recent studies have identified a network of transcription factors that control ageing. What are the regulatory targets of these TFs that control ageing? Identifying these should yield an ultimate understanding of the nature of ageing.

g) Do such processes act solely by controlling levels of molecular damage, or are processes other than damage accumulation primary determinants of ageing?

This goes to the heart of the question of ageing itself. What are needed here are rigorous, functional tests of the role of various forms of molecular damage in ageing. Studies at the organism level are more authentic. Badly needed are better modes of detection and measurement of damage and the reactive species that cause them.

h) What role do mitochondria play in normal ageing?

Mitochondria have traditionally been viewed as central players in ageing, in part due to their production of reactive oxygen species (ROS). Is mitochondrial ROS really a major determinant of ageing? Do mitochondria control ageing in other ways, for example via effects on biosynthesis and/or by regulating processes elsewhere in the cell?

i) Relationships between metabolic aspect of reproduction and ageing: sex differences in ageing.

Correlations between reproductive activity and life span have been seen in many organisms. Nutrients strongly influence fertility, and DR reduces fertility whilst increasing lifespan. In mammals, reproductive hormones, estrogens and androgens, influence intermediary metabolism. How they, with their receptors ER sans ARs, act as determinants of metabolism and ageing is of real interest. In mammalian, ageing gonad activity decreases and lowered estrogenic signaling is associated with altered energetic control, as demonstrated by changes in fat tissues activity and distribution, particularly in women.

“Masculinisation” of fat metabolism and distribution causes a significant, major increase in female susceptibility to metabolic diseases. It is important to understand how reproductive endocrinology interacts with the biology of nutrition to determine overall metabolic effects of ageing. Among other things, this will lead into insights into sex differences in ageing, which are profound, and important for late life human health. A better understanding of sex specific mechanisms involved in energetic metabolism during ageing is needed.

j) Protein synthesis, amino acids and ageing?

Altered protein synthesis has been recently found to have major impacts on ageing. How does this work? What does it mean? Studies of DR are increasingly showing protein rather than caloric content of food to be critical. How does this work?

k) How can our new knowledge of ageing be applied to humans to promote healthy lifespan?

We need to pursue both blue skies work on fundamental understanding, but we also need to pursue interdisciplinary work and work with industry to see applications of our findings to the public benefit.

B.2 How could we design interventional strategies?

Future interventional strategies will be based on novel targets identified by the project including appropriate physical exercise and nutrition suggestions. Current drugs addressing metabolic targets are limited in their application to ageing. However, they could be used as leads for new drug design as a function of results coming out of the study.

3.7 Nuclear receptors

A. State of the art

Nuclear receptors (NRs) are a super family of transcription factors that orchestrate a number of physiological processes trough coordinated regulation of expression of target genes. In contrast to other transcription factors NR responses are mediated by ligands, small molecules that can move easily within the body. This property, ligand-dependent activation, provides NRs with the capacity to respond to cues arising from distant cells or tissues or even the external environment. They may also, via protein-protein interaction, control the activity of signaling molecules in the cytoplasm or in the nucleus. In mammals the NR family has 48 members and includes the estrogen (ER), androgen (AR), glucocorticoid (GR) and thyroid hormone (TR) receptors. However this family is very ancient and NRs are present in all species: this long evolutionary history may partially explain the complexity of action of several of the members of the family. Indeed, it is probable that the first ligand-activated NRs evolved on the basis of their capacity to monitor and regulate first, xenobiotics ans second, lipid metabolism.

Many genetic studies have demonstrated the intimate involvement of this family of transcription factors in the regulation of reproduction, immune functions, metabolism and in pathologies associated with these functions. The major interest in this family of proteins as potential target for innovative drugs has prompted several pharmaceutical companies to develop specific and more selective ligands. However, their use as therapeutic agent resulted to be more complex than predicted and proved the necessity for the field to further progress in the comprehension of the physiology of action even of the members of the family best studied such as the ERs.

Indeed, taking the case of ERs as an example, their function was originally believed as limited to the regulation of reproductive functions. Now we know ERs have a significant metabolic role modulating liver functions and fat functions, in the control of immune functions and central nervous system development and plasticity. Basic as well as clinical studies have underlined a strict association between cessation of estrogen signaling and appearance of pathologies associated with ageing. However, the intervention studies based on hormone replacement therapies have demonstrated our limited ability to restore the physiological functions of the hormone and its receptor in fertile females and indicated the necessity of further analysis of biological rhythms in ER action. Besides, undesired side effects raise the need for further research on targets and more selective drugs, potentially better adapted to the ageing organism, and particularly to age-related diseases (particularly neurodegenerative disease and metabolic disease) both in the periphery (muscle, liver, adipose tissue, bone) and brain.

B. Recommendations

B.1 Understanding the biology of ageing.

- A main area requiring more clinical and basic research is that addressing the relationships between **nuclear receptor control of reproduction, metabolism and longevity.** Ligand-gated transcription, such as that governed by NRs, provides a direct and powerful means to tie environmental and nutrient signals to coordination of metabolism, development, reproduction, and homeostasis, and thereby ageing.
- Nuclear receptor function in protein homeostasis, nuclear organization, DNA repair and mitochondrial function that are intimately linked to **metabolism**.
- As NRs are transcription factors, a particularly important area of investigation in the context of ageing is **genetic control** and switches. Epigenetic modifications of nuclear receptors signaling could well provide the plasticity or adaptive advantage necessary for a healthy lifespan.
- Emphasis should be placed on changes **in ligand availability and therefore the advisability of hormone replacement** during ageing. The major focus of this area of interest covers mainly the ligands for ERs, ARs and TRs as these are the pathways that show the greatest homeostatic shifts during ageing. Of particular interest are the differences between control of ligand availability in different peripheral tissue target (bone, muscle, liver, adipose tissue) and central controls in the brain (specific hypothalamic nuclei).
- This area also includes the roles of nuclear receptors in the relationship between **biological rhythms**, where rhythms covers all biological events with cyclicity, covering the shorter cycles of gene transcription to the longer cycles of circadian rhythms and seasonal rhythms. The latter may not seem immediately pertinent to human ageing but a number of observations underline seasonal changes in human disease patterns, notably depression a major complicating factor in the elderly.

B.2 How could we design interventional strategies?

Interventional strategies in term of hormone replacement therapies are within reach because of the availability of a vast array of synthetic molecules endowed with greater specificity of action. However we underline the need for a better understanding of the cell specificity of action of these ligands and their ability to modulate their receptor activity in time, which mimic the physiological activity of the receptor in healthy, young subjects. A few researchers and pharmaceutical companies are addressing very efficiently this topic integrating bioinformatic analysis of observations based on molecular imaging and systems biology. These approaches enable the generation of innovative algorithms for the evaluation of hormone replacement efficacy *in vivo*.

3.8 Vascular ageing

A. State of the art

Ageing is the major single risk factor for vascular disease. Vascular ageing is characterised by endothelial cells dysfunction and diffuse intimal thickening at the inner surface of blood vessels, arterial wall stiffening, vascular calcification and defective vascular repair. These age-associated changes in vascular structure and function may lead to a number of pathologies including the development of hypertension, atherosclerosis, coronary syndromes, vascular dementia, as well as heart and renal failure.

Organismal ageing and vascular ageing are thought to share common underlying molecular and cellular mechanisms, including those leading to oxidative damage of macromolecules and organelles, DNA damage, depletion of vascular progenitor cells and the accumulation of senescent endothelial and vascular smooth muscle cells. Importantly, these processes are accentuated in the presence of classical cardiovascular risk factors and in vascular pathologies. In particular, most of these molecular and/or cellular events are exacerbated in the context of diabetes and the metabolic syndrome, which in turn are also strongly associated with endothelial dysfunction and with vascular stiffness and remodelling. However, even though the existence of these associations has been widely documented, the mechanistic relationships between risk factors (whether genetic and/or environmental), molecular/cellular events, age-related vascular changes and cardiovascular pathologies are not entirely understood.

The structural and functional manifestations of vascular ageing can be investigated using various non-invasive techniques. These include measures of arterial stiffness, endothelial dysfunction, intimal thickening and calcification. These techniques are very useful to study the relationship between vascular changes in large arteries, the microvasculature and organ damage (heart, brain, kidney and retina). In addition, these parameters are currently being investigated for their predictive value, i.e., to detect vascular changes in young and middle age individuals, in the absence of overt cardiovascular disease.
Currently our understanding of the molecular and cellular events taking place with ageing in the vessel wall stems primarily from evaluation of surrogate systemic markers in specific human cohorts, from post mortem studies and from investigations in animal and cell culture models. In addition, targeted molecular imaging, using isotopes and specific molecular probes, is emerging as a promising non-invasive modality to evaluate in vivo these events. Even though most studies suggest that oxidative stress is the main process driving vascular ageing, the mechanistic picture with all its molecular intricacies is far from complete. Moreover, an emerging theme in this area is the role of attenuating mechanisms, such as those invoked by physical exercise and dietary interventions.

B. Recommendations

B.1 Support research on understanding the biology of vascular ageing, particularly on:

- The role of known molecular/cellular mechanisms of ageing (e.g. oxidative damage, cell senescence) in the aetiology of vascular stiffness and vascular calcification. So far most of the research examining these processes has not studied in detail the relationship between these different phenomena.
- Molecular characterization of signalling pathways modulating vascular ageing. Understanding the influence of gender, smoking, obesity, inflammation and life-style interventions (e.g. nutrition and exercise) on these pathways.
- Identification of protective genetic traits and their relationships to mechanisms of vascular cytoprotection.
- Development of cell and organ culture-based models for studies of cellular senescence, taking into account the relevance and limitations of existing systems.

B.2 Support research that underpins interventional strategies

- A pre-requisite for the design of interventional strategies is the development of better biomarkers (including refinement of existing functional markers) that fulfil some or all of the following conditions: (a) that are “selective” for vascular ageing; (b) that have a higher predictive value; (c) that can be used to indicate when to start interventions and/or (d) that monitor effectiveness of treatments. There is also a need to establish normal range values for different age/ethnic/gender groups with regards to existing functional biomarkers, e.g. for the assessment of endothelial dysfunction and arterial stiffness.
- It is currently unclear how risk factors contribute to cardiovascular disease in the geriatric patient (Framingham score stops at 75 years of age, European studies stop at 60 and then extrapolate; Leiden 85+ study shows that risk factors do not apply to the oldest old). Hence, there is also a need to validate existing markers and/or develop biomarkers which are relevant to the treatment of this population.

B.3 Support development of infrastructures in the following areas

- Non-invasive techniques (including imaging techniques) for *in vivo* analysis of the molecular events that drive the process of vascular ageing.
- Pan European Biobanks such as the BBMRI (Biobanking and Biomolecular Resources Research Infrastructure) for vascular tissue and blood products that include clinical data.
- Animal models, such as the mini pig, that resemble more closely humans with regards to the process of vascular ageing.
- Bio-informatic tools to identify biomarkers that are mechanistically meaningful.

- Bodies that will help to address regulatory/commercial issues currently hindering collaborations and further technological developments in ageing research (e.g. patent issues, current regulations for obtaining ethical consent, tissue storage and to access clinical data are too onerous).
- Longitudinal studies of vascular ageing. Studies could take advantage of existing well characterized cohorts already containing substantial amount of relevant data, including vascular measures, e.g., the Asklepios Study (University of Ghent, Belgium), the Whitehall II Study (University College London, UK), the Interdisciplinary Longitudinal Study of Adulthood (University of Heidelberg, Germany), the SardiNIA/Progenia Project (Italian National Research Council) and the Malmo Studies (University Hospital, Malmo, Sweden).

3.9 Muscle weakness, sarcopenia and physical exercise

A. Overview of the field

Preservation of muscle mass and strength is now recognised as a prerequisite for healthy ageing. Conversely, the loss of muscle mass is associated with a decline in mobility and ability to carry out simple manual tasks which will require day to day help and increased medical attention. Sarcopenia is a universal, age-related, loss of muscle mass associated with a loss of strength and function resulting in muscle weakness. It can start as early as 30 years of age and can result in a loss of about 30-50% of the muscle mass by 80 years of age. Sarcopenia affects 50 million people today worldwide and will affect more than 200 million in the next 40 years. The estimated cost of treating sarcopenia in the USA in the year 2000 was $18.5 billion, equalling 1.5% of total health expenditure. Combating this debilitating and costly condition therefore is of obvious economic benefit and also an increasing priority.

Diagnosing sarcopenia is difficult as there are no standards to define healthy muscle ageing as opposed to debilitating muscle weakness. The current understanding, based on the knowledge generated by previous EU-funded research programmes, recognises 6 major causes and exacerbating factors of sarcopenia: 1) loss of neuromuscular integrity, 2) lifestyle changes such as physical inactivity and poor nutritional status, 3) anabolic resistance to feeding and exercise, 4) hormonal changes, 5) chronic inflammation, 6) reduced muscle regenerative capacity. The increased prevalence of sarcopenia is occurring together with a pandemic of obesity in the population. More than 20% of the population is sarcopenic and obese, giving rise to the condition of sarcopenic obesity (SO), characterised by intramuscular fat infiltration which triggers the release of fat-derived catabolic factors. SO increases the risk of type 2 diabetes and cardiometabolic syndrome.

While sarcopenia is inevitable with ageing, it can be effectively mitigated through physical, nutritional and pharmacological intervention. In contrast, acute trauma or illness often leads to a rapid and potentially devastating additional loss of muscle mass and, as a consequence, loss of independent living. Preventing this transition is a major challenge. In this regard, the impact of communication between muscle tissue and other organs for maintaining health is an emerging field of research.

The regenerative capacity of skeletal muscle is intimately linked to the function of muscle stem cells (satellite cells). These cells can proliferate during periods of muscle growth and repair in response to increased functional demands or injury. The number of satellite cells and their function decreases throughout life. However, the consequences of these changes are insufficiently explored. Old muscle can increase in size with strength training, and it can repair injury, albeit to a lesser extent and at a slower rate. There is some evidence to suggest that systemic and local environmental factors directly influence satellite cell activity; thus, constant exposure to e.g. low-grade inflammation, also related to

intramuscular fat accumulation, has negative effects on muscle regenerative capacity. Manipulation of the environment to improve muscle regeneration and strength should be the focus of future investigations.

It is paramount to encourage interdisciplinary research between physicians and biologists. This will facilitate a translational approach of experimental findings towards development of strategies to treat sarcopenia.

B. Scientific recommendations

B.1 Basic mechanisms and interventional strategies

B.1.1 Understanding and combating sarcopenia and sarcopenic obesity

Understanding sarcopenia and sarcopenic obesity

More longitudinal studies are needed on representative phenotypes of sarcopenic individuals and associated molecular biological profiles. Whereas evidence for anabolic resistance is quite well established, more studies are needed to clarify the roles of muscle protein breakdown, oxidative stress and accumulation of modified proteins in older people. Efforts should be made to dissociate the effects of ageing *per se* from those of disuse and disease. The pathogenesis of sarcopenic obesity needs also to be explored.

Development, testing and implementation of physical, nutritional and pharmacological strategies to combat sarcopenia and sarcopenic obesity

Although strong evidence exists that regular resistive exercise slows down the ageing-associated decrease in muscle mass, its possible role in protecting against the loss of motor units should be further explored.

B1.2 Understanding the effects of exercise, inactivity and inflammation on age-related regulation of skeletal muscle mass and function

Data documenting the physical activity levels in European populations are scarce, leading to gaps in the understanding of the association between inactivity and functional decline with ageing. Studies performed on life-long athletes as well as homozygous twins would be particularly informative.

While inflammation plays a crucial role in the adaptive response of healthy skeletal muscle to exercise and injury, an imbalance between the pro- and anti-inflammatory processes can promote muscle wasting. The molecular mechanisms underlying this process need to be defined.

Since sarcopenia can only be partially reversed with physical exercise, in particular resistance training, in the elderly population, the potential of combining physical activity with additional counter-measures, such as nutritional, hormonal and pharmacological interventions should be evaluated.

B.1.3 Understanding the crosstalk between skeletal muscle tissue and other organs in ageing

Skeletal muscle tissue releases myokines that signal to the muscle cells themselves as well as other organs such as the liver, the heart, the brain, and adipose tissue. However, only a few myokines have so far been identified, and their actions in other tissues are incompletely investigated. Future studies should focus on the pathways of interaction between muscle tissue and other organs during exercise and inactivity, including the identification of novel mechanisms of communication via secreted vesicles (exosomes and microparticles) as well as new myokines and establishing their biological effects.

B.1.4 Understanding functional changes of the locomotor system with ageing through an integrated approach (muscle, tendon, bone, ligament)

Skeletal muscle and connective tissue undergo major biochemical, biomechanical and morphological changes with ageing. However, there is limited information on how these individual changes affect the performance of the locomotor system as a whole. Skeletal muscle wasting is notoriously associated with bone loss; however the signalling pathways associated with this phenomenon are not well understood. Efforts in understanding the role of the extracellular matrix in force transmission and molecular signalling between these structures and how changes in loading affect the regulation of muscle and connective tissue are needed.

B.1.5 Regenerative capacity of skeletal muscle in old age

The number of muscle stem cells (satellite cells) decreases from birth to adulthood. Between 20 and 80 years of age in humans, the percentage of satellite cells will decline from 4-1% of total nuclei in a skeletal muscle. Studies should address the signalling controlling activation, proliferation, differentiation and renewal of satellite cells over the lifespan. In addition, the role of both local and systemic environmental factors on satellite cell function requires further investigation.

B.2 Infrastructure

1. Develop standardised methods of assessing 1) physical activity and 2) functional outcome.
2. Large existing data/sample resources (bio-banks) should be identified and made available for follow-up data collection.
3. Harmonisation of ethical standards for human research, in particular with regard to longitudinal cohort studies and access to bio-banks, across European countries.

4. Support for research exploring gender-specific effects of activity, inactivity and muscle function in the ageing population.
5. Establishment of animal-breeding facility for aged animals and transgenic animals.

3.10 Skin age-related modifications, elastic tissues; and skin biotechnology

A. State of the art

Skin and ageing: the decline of the body Guardian.

To survive and to maintain homeostasis in the hostile environment, higher animals have developed a complex and specialized barrier structure, the skin. The skin is therefore a “functional barrier” that displays thermoregulatory, mechano-sensory (skin is the most innervated organ), biomechanical (elastic properties), immunological, and metabolic properties. Therefore, the maintenance and repair of skin is of the utmost importance. Specific mechanisms of protection, repair and regeneration have been developed, to assume these functions over the entire life time.

Skin ages in response to extrinsic (environmental, such as UV) or intrinsic factors (bad nutrition, stress ...). Wrinkles are among the most visible signs of ageing. This has a tremendous social impact, especially for women after menopause, a major gender issue. Wrinkles reflect deeper modifications, with important consequences for the skin functions, in relation with the decline of immune, cardio-vascular, central/peripheral nervous or endocrine functions and mechanisms of regulation. For instance, skin, as other parts of the body, is susceptible to the consequences of a bad nutrition (excess sugar and fat) or diabetes. This results in the accumulation of oxidants that may have deleterious effects on cutaneous mechanical and repair properties.

The prevalence of chronic wounds (with impaired repair) is highly correlated with age. Recent surveys of European hospitals show that around 1 in 5 patients have a pressure ulcer, which affects their quality of life and should be avoidable. Altogether, the population prevalence of wounds is 3-4/1000 people (estimated at 1.5-2.0 million in the EU), with an annual incidence of 4 million individuals, affecting mainly the elderly and frail/disabled persons. While research on wound healing is well developed, the investment for developing new models mimicking chronic wounds for the elderly and for providing better treatment to them is by far not enough supported.

Non-melanoma skin cancers (NMSC) are by far the most frequent tumors in humans with two important risk factors, UV and age. Therefore, due to the ageing of the European population, there will be a notable increase in the number of skin cancers to be diagnosed and treated. The development of new tools for early diagnosis in research models and during therapies is a major issue of future research, for all ages but particularly for the aged population.

The decline of the defence capacity against pathogens is clearly associated to a loss of the functional barrier efficiency (recently called “dermatoporosis”), although poorly investigated.

Researchers in dermatology have developed sophisticated studies on stem cells and progenitors, and on in vitro models. These are the so-called unique skin equivalents, which allow to explore the many fold inherently complex functions of the skin and their regulation. These tools are respectful of ethical issues, which recommend reducing the use of animal models. Worth to note however, animal models are already available, mainly from other domains of research, and should be investigated in the light of skin ageing.

Beside the recent development of imaging tools that allows visualize deep layers of the skin, other developments are in progress to evaluate/quantify the different functions of skin, such as its biomechanical properties which are clearly among the best biomarkers of ageing. Therefore, the industry selling/using diagnostic tools can strongly be benefited by a focused effort on the research on skin ageing, both by developing new tools or by miniaturizing actual tools that could be brought to the patients. This will attract further other companies specialized in nutritional supplementation, cosmeto - dermato – pharmacology or fillers.

B. Recommendations

Research and development in the area of basic and applied aspects with respect to skin ageing should engage distinct areas of work, which is to investigate specific biological mechanisms and clinical age-related manifestations. Particular emphasis should be given to:

- the natural complexity reflecting the interdependence of various cellular entities embedded in this tissue and organ;
- the functional properties of tissue-borne stem cells in conjunction with their respective niche and its microenvironment with respect to regenerative vigor and repair capacity;
- age-associated alterations and pathologies related to functional topical changes of the immune, vascular and neuronal system.

Comprehensive research encompassing three major interrelated topics are envisaged:

B.1 Biology of ageing skin

Intrinsic and extrinsic stressors and impacts on skin integrity have been extensively studied in cell cultures using single cell types. Besides that, alterations in skin have been documented in a broadly descriptive fashion applying histological techniques. Both lines of research should be combined in future approaches by:

- implementing valid in vitro models representing the complex anatomical nature of skin;
- specifying the functional decline of stem cells within the dermis and epidermis in respect to interactions with their niches as well as their abilities of sensing molecular determinants that are contained within the microenvironment ;
- developing skin equivalents for the refinement, reduction and replacement of animal research models;
- generating or implementing suitable animal models amenable for evaluating human age-associated skin alterations and pathologies.

B.2 Translational clinical research

Overwhelming clinical evidence shows that skin ageing is the main driver of age-related pathologies such as the increase in susceptibility to infections or chronic and pressure ulcers. Also the prevalence of non-melanoma skin carcinoma increases with age with its single most important extrinsic determinant, photo ageing. These distinct topics should be addressed by the following means:

- employment of standardized 3D models which are specifically designed to mimic situations which result in pathologies such as NMSC ;
- standardized protocols for documentation of ulcer development;
- identification of drug-able targets to balance skin-specific immunological responses;
- research on distinct animal models defined in contemporary ageing research;
- implementation of interventions on gender-based alterations of skin.

B.3 Diagnostic measures, tools and instrumentation

Skin research and clinical interventions is particularly benefited from the possibility that non-invasive technology can be established for the examination of functional properties of skin using commonly available technology. It is anticipated that further advancements in confocal microscopy as well as Raman spectroscopy could be accomplished. Particular emphasis has to be put on the miniaturization and portability of such diagnostic tools and their applicability in a clinical setting. The following dedications with respect to skin ageing and age-associated pathologies are considered most important:

- Examination and quantitative enumeration of precancerous modifications;
- Evaluation of vascular performance in skin;
- Visualization of innervation and functionality thereof;
- Estimation of skin ageing with respect to integrity and functional decline – dermatoporosis;
- Comparative analysis of skin functionality with respect to the performance and integrity of other inner organs.

B.4 Infrastructures

The achievement of the proposed measures necessitates the following **infrastructural innovations**, field-specific approaches, technical progress or cooperative strategies:

- Skin is accessible and biopsies for both pathological and healthy tissues can be collected, even for a follow up of healthy ageing, in contrast to other organs. This makes this organ unique. Biobank containing age-specific as well as pathology–associated skin cells and tissues can be set up much easier than for other organs;
- Prospective longitudinal study (interventional) addressing gender-based differences with respect to skin ageing (menopause/andropause; sex-specific anatomical features; photo-ageing/photo protection);
- Establishment of novel (miniaturized/portable) imaging technology;
- Compilation of ethical standards for collecting biopsies on a European-wide scale, their use for academic research purposes as well as a possible sharing of the material in commercial applications.

3.11 Clinical Biogerontological studies

A. State of the art

The field of clinical biogerontological studies represents a major challenge for our future endeavour into better understanding pathophysiology of the ageing process. By aiming our research at both cellular and patient level in a co-ordinated fashion, ways to render correlates of ageing and related diseases amenable to intervention could be discovered and strongly impact upon the well being of the ageing population, as well as on specific diseases of the elderly.

One of the major strengths for focusing on clinical studies in the ageing population, stems from strong epidemiological trends, reflecting ongoing demographic changes with steep increase of the number of the oldest old in the next decades. In addition, available expertise and technological advances have now matured into highly sophisticated tools that could prove valuable in analysis of findings from large clinical and biobanking databases.

Growing interdisciplinarity in geriatrics and biogerontology also holds much promise as greater emphasis is placed on ageing research, enabling fruitful interaction between basic scientists and clinicians. There is increasing interest into creating a much more attractive environment of collaboration between these two fields. Such a productive interrelationship could greatly augment novel findings at molecular and cellular level being transferred to an integrative clinical approach to the patient. Special attention should be placed into promoting networking opportunities and enhancing exchange capabilities within and between European countries, characterized by multicultural national diversities.

An inherent difficulty in many ongoing clinical studies is not just exclusion of the very old, but especially of those in frail and/or with complex comorbid conditions. This should be corrected, since it might be a limiting factor towards generalising findings and fail to reveal those mostly at need for evaluation and intervention.

One very important aspect of dealing with clinical biogerontological research is current lack of uniform trial guidelines and ethics recommendations, mainly attributed to great legislation differences between countries, which pose a serious obstacle when dealing with study design, patient recruitment, handling of samples and data acquisition.

Existing educational schemes in both molecular biogerontology, ageing biology and clinical geriatrics are individually robust and productive. However, increasing collaboration and definite goal setting between biologists and clinicians should greatly improve their common research potential.

We have set up a list of attainable objectives that could pave the way into bridging the existing gap between basic research data and a comprehensive approach of the old subject. By structuring such a link, we could vastly improve our understanding of both the healthy and disease-related ageing processes, as well as formulate rational diagnostic assessment tools and therapeutic modalities.

B. Recommendations

B.1 Identification of established research structures and themes

Relevant research lines proposed

- Biomarkers of frailty (clinical-biological risk factors for functional decline, biological determinant mechanisms for frailty) in order to identify those subjects at risk and to propose interventions to reverse the process.
- Nutritional and/or exercise studies combined with biological indicators sensible to changes.
- Biological mechanisms and biomarkers of frequent geriatric syndromes (delirium, falls) and intervention studies in accordance.
- Studies in frail dependent institutionalized patients to prevent/treat sarcopenia and/or pressure sores.
- Studies oriented to improve the responsiveness on vaccines in older adults and the oldest old.
- Biological counterpart studies in existing European cohort studies (SHARE).
- Diabetes and pharmacology counterpart.
- Need for a consensus short list of biomarkers of ageing to add to the clinical description of the study subjects.
- Innovative research themes should not only be designed to address a challenging biology question but be also clinically driven. They should aim at bringing together topics of paramount interest to the biologist and at the same time meaningful to the clinician.
- In addition, in the area of preventive medicine, much could be gained by creating a common interest frame of research activities for both biologists and clinicians.
- Clinicians and biologists need to be regularly informed at professional level about opportunities for expanding their own research projects and accessible domains to aid them find synergistic initiatives and connect with existing structures by topic. Frequent exchange of ideas and findings should be promoted through networking, regular meetings and presentations. Such a platform of scientific communication on ageing is currently lacking in Europe.
- Implementing new developments in the areas of genomics, proteomics, metabolomics etc, in ageing research should be encouraged. It would lead to our comprehensive appreciation of fundamental regulation of age-related biological processes. Such an AGEnOMICS (AGE + …OMICS) initiative could encompass ageing system biology and physiology along with clinically applicable indices of disease prediction or response to treatment.

- Organ decline and morbidity in ageing often leads to broader psychological sequelae and socioeconomic burden and vice versa. Reinforcing links between psychosocial and biological sciences pertinent to old age should become one of the priorities in the near future.
- Emphasis should be placed on the enormous potential social impact of clinical biogerontological studies upon issues ranging from avoiding general misconcepts about ageing, benefiting from interventions created to promote overall well-being and quality of life, as well as getting accustomed to changing demographic trends. The society at large, especially health professional and social services, should be prepared for and readily adapted to the challenges raised by the results of clinical biogerontological research.

B.2 Promoting recruitment of older persons for clinical biogerontological studies.

- There is an urgent need for standardized geriatric assessment to be used in clinical biogerontology research, so that old subjects could be exquisitely characterized. Harmonization of relevant legislation pertinent to clinical trials and studies in the old population, across European countries, is also required. It is crucial to promote studies designed for 'real patients', without extensive exclusion criteria that rule out the frailest ones. The use of validated classification schemes (ICF) and/or assessment tools (GerontoNet or RAI-MDS) should be encouraged.
- Uniform ethical standards and considerations should be drafted. As research develops further, new questions arise, ranging from elementary issues related to allowed sample collection and modes of conservation, up to storage requirements, handling and long term utilisation. Informed consent guidelines currently do not correct for dissimilarities between countries. Standard Operating Procedures and an appropriate European Code of Ethics for Clinical Biogerontological Research should be created.
- Long term, large scale, strategically designed longitudinal studies are of utmost importance for promoting clinical biogerontological studies. This is particularly relevant to the European countries, where population heterogeneity and environmental diversity has been shown to influence the ageing process and play a critical role in disease prevention. These studies should be conducted by experts in the field, enabling acquisition of specimen and sample panels in a co-ordinated fashion. Thus there should be greater benefit from sharing data from BioBanks and registries, easier access to hard-to-find patient samples including frail ones, and systematic analysis of larger amounts of data.
- Outmost quality control population should be clearly defined in Clinical Biogerontological studies, not only between health and disease, but also across the age span as an age-dependent correlate to the appropriate question.
- Safety should be of great importance in Clinical Research for the old population. Study design should strictly focus on specific objectives and every effort should be placed in ameliorating dangers from exposure of subjects to malpractice or drugs. Understanding the concept of "pharmacosenescence"

and applying relevant modelling techniques might offer an alternative in selection of the appropriate subjects.

- Dissemination of ageing-related research is not just an issue of the scientists or clinicians. The old population at large should be sensitized to the impact of such research and encouraged to actively participate in educational activities, e-learning and exchange of information in regards to disease prevention or therapy via public health initiatives, patient groups and associations.

4. Needs in term of training, networking and dissemination

4.1 Networking

Over the last 15 years, several successful communication networks / co-ordination actions have been sponsored by the European Commission (e.g. MOLGERON, LINK-AGE and WHYWEAGE), which proved extremely helpful to the growth of this field in Europe by enabling **personal contacts and scientific exchange**. In the future, **extension and widening of these networks** will be necessary in order to tend to more multidisciplinarity and to include e.g. clinicians, mathematicians and bioinformaticians. Close contacts between these disciplines are a prerequisite for establishing "systems biogerontology", a subfield of ageing research able to tackle the complexity of ageing in all regards. WHYWEAGE has initiated the integration of experts from various fields and clearly further extensions of this process must go on. In addition there is a need to attract new teams in ageing research and to train more young scientists so they can be included in ongoing research and most importantly will build up the future research developments in the field.

One proposal to build capacity and encourage "discipline hopping" is to establish a virtual **European College on Ageing**, which would have the capacity to issue small pilot grants in the subject area and to support training and networking activities. The establishment of such a European College on Ageing would improve cohesion, encourage networking, facilitate collaboration, multidisciplinarity and increase visibility. Such a "College" would also provide a central point of reference and **co-ordination of both basic and clinical research** on ageing in Europe and would bring together the most outstanding investigators, delineate controversial aspects in research and publicizing consensus statements on important public health issues pertinent to health or disease-related ageing. Biogerontology is an opportunity for geriatric medicine and becomes an attractive domain for high-level researchers, as reflected by the impact factors of journals dedicated to ageing. Several research themes have the potential to bridge the gap between clinicians and biologists.

Moreover, standardization of basic commonly applied methods should be broadly discussed and communicated through established learned societies and implemented in good-clinical research and care taking practices. For this purpose **specialized networking programs** should be established.
We also need to promote **networking activities between areas with significant potential for synergism**, where traditionally there has been little or no interdisciplinary activity, e.g., the connection between vascular ageing to ageing of other organs such as skin, kidney and brain; rare disorders that affect the vasculature e.g. progeroid syndromes and connective tissue disorders such as Ehler Danlos disease, Marfan syndrome and cutis laxa. Given the importance of diabetes and the metabolic syndrome in vascular ageing, develop interactions between workgroups on Ageing and Diabetes (e.g. cross link between DIAMAP - Road Map for Diabetes Research in Europe - and WHYWEAGE.)

Last but not least, we should support **networking between members of relevant European learned Societies** (ex : European Society of Cardiology, European Atherosclerosis Society and European Society of Hypertension) and scientists undertaking research on ageing in its different forms (members of LINK-AGE, WHYWEAGE, etc).

4.2 Training

Training programs for young scientists starting already at **bachelor level** should be on the agenda of biogerontology networks in order to attract many more young scientists to the field. Ageing research requires specific and profound knowledge in human biology, medical terminology, biochemistry, molecular biology, statistics and bioinformatics. Therefore it should be of high priority to give targeted training to young medical doctors/nurses, master/PhD students in Biology and master/PhD students in statistics and bioinformatics in the respective complementary fields. Familiarity with both basic and clinical biogerontology as a component of studies for both graduates and post-graduates of biological and medical disciplines is of great importance for the future of the field. This could be implemented in close dialogue with existing and emerging master programmes at various places in Europe. Since a few years, specific Master programmes dedicated to the Biology of ageing and longevity have been launched in different European universities (Aarhus, Athens, Jena, Lodz, Namur, Paris, etc.). However, these are quite recent initiatives that should be pursued and merged to set the basis of a **European Master on ageing** that would be beneficial for high level training and visibility to attract the best students.

The European College on Ageing mentioned above would also have a training focus and a strong multidisciplinary ethos. Its activities could include summer schools and eventually a Euromasters in Biogerontology. Training is likely to have a powerful influence - to encourage interdisciplinarity it first has to be valued by the individual disciplines. As an example, for immunologists, the link to medicine is already strong, but the link to social sciences is less obvious. There is a good evidence base for the influence of psychosocial factors upon immunity and if this is introduced to researchers in their training they are more likely to consider broad influences and seek interaction with researchers from other disciplines. Actions to **encourage multidisciplinarity in training** and also **support cross discipline sessions at conferences** would help greatly.

Exchange programs should be encouraged. Promising young students and investigators must be helped. Common and high quality educational schemes across Europe, especially focusing on higher degrees (Masters and PhDs), implicating interdisciplinary collaborations should be encouraged in EU calls. Uniform criteria for Geriatric Practice and accreditation, fellowship certification and post-doctoral

programs of specialist training should be highly advertised and supported. Attractive physician-scientist pathways placing a special focus on long-term commitment to Gerontology should be prioritized. Besides the public awareness, the vested interest of medical doctors with respect to specific gerontological questions in particular underlying common mechanisms of skin ageing should enhanced and concerns regarding innovative interventional strategies have to be addressed accordingly. Upcoming knowledge regarding basic mechanisms of ageing biology in general and gender-based peculiarities in particular should be incorporated in the curriculum of university and residential training programs of medical doctors. In general, the level of knowledge of biogerontology among the medical profession is dismal.

Maintaining training and teaching in key areas is a concern as there is shortage of scientists with expertise in “classical” physiology, concepts and techniques that are going to be increasing required if the full impact of the complexity of signaling in the whole organisms is to be appreciated. Examples could include effect of gluco-corticoids used as anti-inflammatory agents and their interactions with PPAR and LXR signaling and hence their effects on general metabolic responses in the periphery (liver, muscle, adipose tissue) and the homeostatic set-points in the hypothalamus. Scientists with expertise in comparative biology are needed, particularly with training that spans genomic and whole animal physiology.

Training in systems biology. Data generation is increasingly automated and requires complex analysis using computational tools. There is an increasing need in all areas of biology for more researchers with computational skills - biostatisticians, computing scientists, modellers, data analysts, database experts. For any scientist to apply a systems biology approach two essential skills are required: first, understanding of quantitative methods, and second, ability to work collaboratively across traditional academic disciplines.

Training is urgently required at all stages of the scientific career. At the earliest stages of training there must be more thorough grounding in quantitative methods. Early career scientists must receive training in working effectively across disciplines. There is a requirement for 2nd discipline training for late career scientists.

No physical location has all of the necessary expertise for the full range of systems biology and networks and staff exchange are essential.

Systems biology is well established with many centres of excellence across Europe. Specific expertise in Ageing has been assisted with national funding e.g. DE (GerontoSys), UK (CISBAN).

Summer schools and workshops represent important opportunities for meeting and stimulating new research ideas. Summer schools targeting students and early-stage researchers would be beneficial.
We also must get possibility of **support for young researchers who are about to reach independence** (tenure track positions, *cf.* Wellcome Fellowships), *e.g*. by increasing funding for young investigators through ERC. Industrial co-sponsoring of these activities needs to be encouraged.

4.3 Dissemination and communication

As immediate measure, it is recommended to organise a **workshop(s) on longitudinal studies** with the focus on design of the studies, sampling strategies, and dissemination of the results. Inviting scientists that have already successfully performed longitudinal studies will enable planning of integrated future studies and will promote interdisciplinary collaborations.

Moreover, we strongly recommend the organization of **surveys and conferences** that will aim to present to other scientists who are not yet members of an existing ageing network, the concept of ageing and its importance. Multidisciplinary conferences should aim at bringing together ageing researchers with researchers from other disciplines, the most obvious being those working on age-related and degenerative diseases.

In order to support the already ongoing and here suggested European communication efforts, basic and pro-active dissemination of information and public relation should be further promoted by **connecting national societies of gerontology and geriatrics ideally in all European countries with other societies such as biochemical or medical societies**. This will be possible by aiming at the presence of biogerontology board members to other societies. In addition, it is desirable to create a **European society of biogerontology**. Clinicians and particularly geriatricians should put the biogerontologists and their scientific associations in contact with the European geriatric scientific societies, and stimulate their participation to the clinical scientific meetings to present their researchs: European Union Geriatric Medicine Society (EUGMS) which represents all the national geriatric societies in Europe; International Association for Gerontology and Geriatrics IAGG, European Clinical section; Promote Clinical Biogerontological research in the European geriatric journals; Age and Ageing, Ageing clinical and biological research, Journal of nutrition health and ageing, European Geriatric Medicine.

5. Industrial involvement, industrial relevance and social impact

Europe hosts many excellent scientists in the field, however, this is not matched by adequate funding, **especially in view to the fact that ageing and accompanied age-related functional decline is the major challenge of European healthcare systems**. Since ageing is a primary risk factor for multiple disorders and generalised frailty, **multiple stakeholder groups** are potential beneficiaries, including scientists, the population, policy makers, industry and practitioners. The BBSRC-MRC IPSOS-MORI in the UK provides a potential example of **public consultation**. Other stakeholder groups who should be consulted include the legal profession. Consultation with National Government/state authorities through their standard routes (e.g. select committees) will become an increasingly important responsibility as this area develops. The primary need of all stakeholder groups is the provision of objective scientific data on the biology of ageing.

Stakeholders **The whole population**

Ageing affects the organism systemically and constitutes the principal risk-factor for major pathologies, which in projection will affect tens of millions of individuals in the developed world (Centers for Disease Control and Prevention, www.cdc.gov/aging/pdf/saha_2007.pdf). Given these premises, it is clear that Europe would draw enormous benefits from **treatments aimed to slow down ageing** and therefore to improve the general health status of old people.

While no such treatments are available so far, there is progress in basic biology, which holds the promise to develop efficient intervention aimed to improve health span and quality of life.
For example, research in immunosenescence will provide data on how to maintain a healthy immune system, including data on lifestyle and medical interventions (e.g. vaccination). As immunity (inflammation) impacts upon other conditions, these data are relevant to health and independence overall.

Biomarker research could also play an enabling role in the field of assistive technology, which is currently hampered by a lack of understanding of what biological factors promote or prevent withdrawal from independent living. The combination of biomarkers research and new technology could enormously promote the **quality of life at old age**, simply by effectuating the necessary treatment at their own homes.

Successful interventions in ageing with the aim of reducing levels of ageing-related disease will have profound social impacts, both positive and negative. While the positive impact, particularly the necessity to improve health of women in the last years of their life have been described, here it is important to view possible negative impacts in the contexts of the gains in terms of reduced disease and improved health.

Broad consultation with the public, and improvement of public awareness of where biogerontology is crucial, will ensure good impacts of public support of science and healthcare policy.

Stakeholders Industries

European biotechnology and pharmaceutical industry currently do not undertake significant activity in this field. In part this is due to a lack of understanding by regulatory bodies that the ageing process can have a significant impact on the trajectory of age-associated diseases. This road map will help to address this issue by identifying molecular and cellular pathways amenable to pharmacological or life-style interventions that will delay/revert the alterations associated with ageing. In addition, the development of animal models that reflect human ageing will facilitate pre-clinical studies (example: vascular ageing). The involvement of industry in developing preventive measures is complex, also because of critical aspects such as intellectual property issues. However, we recommend encouraging interactions with Big Pharma, as we are confident that in the next decade preventive medicine will gain corporate interest.

The ageing biomarkers field offers **commercial opportunities**, with respect to the production of tested and validated kits for biomarker measurements. Undoubtedly, the biology of ageing is traditionally an area of weak market pull, *i.e.* outside the scope of pharmaceutical industry. This is due to the lack of an enabling **legal environment** (e.g. ageing is not recognised as a disease entity), which should be revisited in the light of new knowledge about ageing. **Functional foods** may provide a better area for immediate market entry.

Clinical Biogerontological Research should attract Pharmaceutical and Biotechnology companies active in vaccines, nutrition, medical devices and diagnostics, since there is huge potential for such products for human use to be applied in the older population.

Research in immunosenescence can provide the evidence base for their products that claim to improve immune health and, in particular, would determine if this was relevant to older adults (pharmaceuticals, nutriceuticals). Most new drugs are not trialled in older adults and this is a major issue that needs to be addressed in planning clinical trials.

Skin cancer is an important issue for pharmacology companies, while other companies are highly concerned by caring wounds or burns, especially at the bed level in hospital or for disable persons. Beside, a better knowledge on aged skin can improve the efficiency of local vaccine. Skin ageing is obviously also the major interest of dermato-cosmetic companies. While new devices or new

standardized methods are still required to replace toxicology assays or to test the efficiency of treatments on skin.

It may also help to increase the involvement of the well-being industry (sports and foodstuffs) in scientific research, mainly through collaboration with academia and other science-based organizations, in order to develop better/sound health-promoting products.

Nuclear receptors are amongst the most ‘druggable ‘ molecular targets with enormous potential for providing new drug targets and /or enabling more appropriate use of current ones. Nuclear receptors are the second largest category of drug target with an estimated market of 50 billion Euros in 2003. They are obviously of major interest to pharmaceutical companies as prime targets for new approaches to many diseases. Corticosteroids for GRs (e.g. targeted in inflammatory disease) and thyroxine (a TR ligand representing >2% of all prescriptions) are among the most commonly prescribed drugs, but each has drawbacks and greater response and target selectivity is continually sought. Increasing detail of molecular structure fro m X-ray crystallography, information on potential for ligand binding, in silico chemistry and systems biology applications are continually expanding theoretical and practical frameworks for testing novel drugs.

There are many ways in which the market can play a role in interventions that enhance healthy ageing, including the following. 1) The development of pharmaceuticals and nutraceuticals that impact ageing (for example, hormone replacement terapy as well as those that mimic the effects of dietary restriction). (2) The development of improved nutritional regimens for better health, involving the pharmaceuticals industry. (3) The development of new exercise regiments involving new exercise tools.

There is great potential for systems biology to contribute to the development of novel ways to intervene in the ageing process, of benefit both to industry and to citizens of European society. For example, there needs to be the identification of target within networks most amenable to beneficial modification. There also needs to be better methodology to assess the effect of interventions within networks, i.e. biomarkers. This development will require interaction between many different groups with different expertise, which is often difficult to find outside the university setting. There is ample opportunity for industry to buy into this expertise and help steer its direction. Systems biology is now sufficiently mature for industry to make rapid low risk gains. Much advanced software is being developed but there is need for bringing this software up to industry standard in terms of documentation, reliability, maintenance and support.

Although the field of protein modification, maintenance and oxidative stress in relation with ageing is mainly represented by academic research, there is a growing interest in the industry, e.g. pharmaceutics, cosmetics and food industry as well as upcoming biotech companies. That is highlighted by ongoing collaborations between medical and scientific university departments and industries. The research community, including young researchers whose career development will be impacted, will be the primarily beneficiaries from the increased knowledge and further development of new technological approaches aimed at tackling the complexity of fundamental issues in the field of protein modification, maintenance and oxidative stress in relation with ageing. The industry will also benefit from the outcome of such research to improve healthy living, to set the basis for the identification of the effects of existing products (especially for the so-called anti-oxidant active compounds), and to develop new products including those of pharmacological relevance. Industries that are most likely to get benefits include pharmaceutics, cosmetics and food industry.

Stakeholders **Health care professionals**

Public health policy makers will be interested in the preventive options oriented to healthy ageing and the prevention of functional decline in the frail elderly.

Ageing research in general has considerable utility to health care professionals working in preventive disciplines but must be logically coupled to treatment options. As an example, literally everybody will benefit from biomarker research. The results of this research can be applied as diagnostic and predictive biomarkers, which will help identify people at risk for the early development of age-associated disease. Intensified intervention in this group of people (life style changes; nutritional interventions; treatment with established or novel drugs) will contribute to healthy ageing at large, and society will benefit from the socio-economic advantages of this progress.

Research in immunosenescence will provide the evidence base for improvements to encourage immune health in old age, this could be lifestyle or pharmaceutical.

This road map can also help in the development of medical devices for easier and more reliable detection of vascular ageing. For example, this would provide benefit to clinicians by enabling earlier detection of arterial stiffness in the population at large so that more timely interventions can be implemented. It will influence policy makers (governments) and health care providers to alter public behaviour in order to decrease the incidence of age-associated diseases. It may also contribute to the reduction in health care costs without compromising quality of provision

6. List of abbreviations

NR	Nuclear receptor
NMSC	Non Melanoma Skin Cancer
GeHA	Genetics of Healthy Ageing
mtDNA	Mitochondrial DNA
ROS	Reactive Oxygen Species
DDR	DNA Damage Response
GO	Gene Ontology (standard)
SNPs	Single Nucleotide Polymorphisms
IRP	Immune Risk Profile
DR	Dietary Restriction
TF	Transcription Factor
ER	Estrogen Receptor
AR	Androgen Receptor
HDL	High Density Lipoprotein
GR	Glucocorticoid Receptor
TR	Thyroid Hormone Receptor
BBMRI	Biobanking and Biomolecular Resources Research Infrastructure
SO	Sarcopenic obesity
ICF	International Classification of Functioning, Disability and Health