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Translational strategies in aging and age-related disease

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Abstract

Aging is a risk factor for several of the world's most prevalent diseases, including neurodegenerative disorders, cancer, cardiovascular disease and metabolic disease. Although our understanding of the molecular pathways that contribute to the aging process and age-related disease is progressing through the use of model organisms, how to apply this knowledge in the clinic is less clear. In September, *Nature Medicine*, in collaboration with the Volkswagen Foundation, hosted a conference at the beautiful Herrenhausen Palace in Hannover, Germany with the goal of broadening our understanding of the aging process and its meaning as a 'risk factor' in disease. Here, several of the speakers at that conference answer questions posed by *Nature Medicine*.

What are we aiming for in terms of 'treating' aging? In a clinical trial, should we measure longevity or healthspan? How do we overcome the fact that some endpoints related to aging are not recognized as 'diseases' by

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regulatory bodies, and what clinical endpoints are tractable targets in early proof-of-concept trials?

Rafael de Cabo: We should focus on developing interventions that increase the overall well-being of our aging population in terms of physical, psychological and social health factors[1]. Because most of these conditions occur more frequently in older people than in younger people, we hypothesize that aging is a driving force of pathology in old age that is best characterized as multi-morbidity[2]. It is imperative to design trials to test interventions that can delay the onset and progression of multi-morbidity and the phenotypes of aging, such as osteoporosis and sarcopenia, and not to focus on one disease at a time. Studies in model organisms have clearly demonstrated that it is possible to identify simple interventions that can postpone the onset of multiple chronic diseases. The time has come to initiate clinical trials that measure tractable phenotypes and well-established biomarkers (i.e., gate speed, pulse wave velocity, and circulating interleukin-6 levels, among others) that predict good survival outcomes and ameliorate the onset or progression of phenotypes linked to chronic diseases[2,3]. However, there is a lack of information regarding the onset and progression of chronic diseases and aging phenotypes in longitudinal studies of aging in mice and other model organisms. The development of longitudinal assessments of aging phenotypes in multiple model organisms, with attention to differences in sex, strain and diet composition, could accelerate our ability to better screen for interventions that would lead to people living longer and healthier lives.

Joan Mannick

Joan Mannick: Healthspan, rather than lifespan, should be measured in clinical trials of anti-aging therapeutics. Because aging is the major risk factor for most chronic diseases, therapies targeting aging pathways are likely to increase healthspan by ameliorating multiple aging-related diseases. Moreover, several conditions that substantially affect the healthspan

and quality of life of the elderly (such as mobility disability) are not currently recognized as diseases by regulatory bodies. Therefore it will be important to work with regulatory agencies to define clinically important phase 3 registration endpoints for these conditions. Tractable clinical endpoints in early proof-of-concept trials are endpoints that can be measured in a relatively short time frame with relatively small patient numbers, and which predict clinical efficacy in larger, more expensive phase 3 clinical trials. For instance, vaccination response can be used to assess immune function in proof-of-concept trials for therapies targeting immunosenescence (the decline in immune function that occurs in the elderly and leads to increased susceptibility to infections). Demonstration that a therapy improves vaccination response in the elderly with an acceptable safety profile 'de-risks' testing the therapy in later-stage clinical trials with clinical endpoints (such as decreased infection rates) that require larger and longer clinical trials for proof of efficacy.

Linda Partridge

Linda Partridge: We have learned from experiments with animals that modulating the activity of pathways involved in aging can provide simultaneous protection against multiple age-related conditions. The strategy in 'treating aging' should thus be to target similar mechanisms in humans to produce a broad-spectrum preventative medicine against aging-related disease[1]. The tactics for achieving this goal will need to address the financial and regulatory issues involved in running clinical trials for anti-aging therapies. A population-wide trial with such a broad clinical outcome would be not be accepted by regulators and would be prohibitively expensive. Small-scale clinical trials on short-term outcomes relevant to specific diseases, which would hence be both affordable and recognized by regulatory bodies, would allow incremental progress. Monitoring subjects in these clinical trials for other beneficial health outcomes would provide candidates for future clinical trials for other conditions.

Jan van Deursen

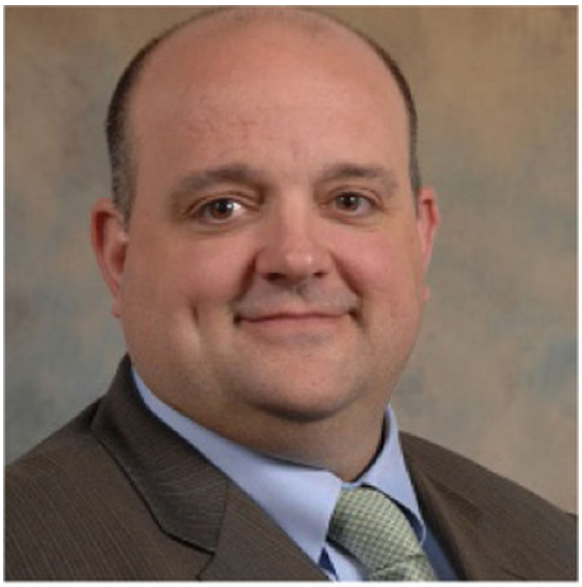
Jan van Deursen: I think that making our stated research objective the discovery of interventions that positively influence healthspan aspects of aging in humans, rather than those that extend lifespan *per se*, will be met with less skepticism and fear of pathologizing the normal aging process by both prospective patients and regulatory agencies. The fact that increased healthspan often correlates with lifespan benefits in animal models is a fortuitous bonus that may or may not be relevant to, or desired by, patients. Moving forward into clinical trials with this mind-set, it is sensible to measure both functional parameters that directly influence quality of life, such as mobility and strength, as well as 'hard' endpoints, such as age at death. A good 'firm' measureable endpoint that has plausible predictive value for both quality of life and lifespan is delayed emergence of new diseases. This approach is gaining traction, as demonstrated in the recent success of Nir Barzilai and others in convincing the FDA to use delay of new disease onset as a surrogate indicator for healthspan extension. I think this is a step in the right direction, and that it may ultimately end up serving as a cost-effective template for future anti-aging clinical trials.

Saul Villeda: By 'treating' aging the goal is to extend healthspan. The ideal would be to preserve all the faculties of a young person in their prime in an old person and maintain them for as long as possible, ideally well into a person's golden years. From a clinical trial perspective, rather than defining aging itself as a disease, we could assess tractable responses to common environmental insults that are readily observed in the elderly. For example, we could preemptively recruit people to trials, and then we could track a function such as regeneration by observing and measuring skin wound healing after common types of injuries in the elderly for which clinical treatment is sought. Alternatively, something such as cognitive reserve could be measured in elderly populations that exhibit post-operative cognitive dysfunction[4] when undergoing routine surgeries, such as hip replacements. The idea of such an approach would be to take advantage of situations in which elderly

individuals are already in a defined clinical setting for a very specific situation and in which assessments of individual functions are available. This would also allow for the exclusion of individuals who are already exhibiting ongoing chronic disease.

What types of markers should thus be measured in a clinical trial? How do we account for the heterogeneity of the human population with respect to these markers?

Mary Armanios: As Richard Pazdur, the director of the United States Food and Drug Administration's Office of Hematology and Oncology Products, was recently quoted[5], "The primary endpoint of any trial should be the patient." The risk of using biomarkers as endpoints is that their alteration does not necessarily equate to clinical benefit. There are additional challenges with the standardization and reproducibility of biomarker measurements, defining their normal ranges in the population, and understanding the thresholds associated with disease. In the area of telomere biology, there is a recently appreciated caveat that may also be emblematic of other biomarkers of aging. Telomere shortening is one of the best-characterized mechanisms of cellular aging, and, at first glance, it may seem that the more telomere the fitter the individual. However, there is emerging evidence suggesting that abnormally long telomeres predis-pose to certain cancers, such as cutaneous melanoma[6]. The fact that there may be delicate balances in the regulation of the biological pathways involved in aging makes it even more critical to design trials that have patient-centered endpoints.



Rafael de Cabo

Rafael de Cabo: Despite the genetic heterogeneity in humans, there are a growing number of well-defined and well-characterized age-associated biomarkers that are derived from observational studies. In most cases, these biomarkers are gathered from minimally

invasive protocols and are easily collected longitudinally. Multiple epidemiological cohort studies have concluded that walking speed is perhaps one of the strongest predictors of well-being and survival in humans, and that it also reflects health and functional status at the individual level. In an effort to accelerate the discovery of translatable biomarkers that represent common aging phenotypes across taxa, investigators in the Trans-NIH GeroScience initiative have discussed and published their initial consensus to create synergy between bench scientists and clinicians[7]. In this context, there is also a growing interest in including measurements of resilience (for example, the ability to mount an immune response to an immune challenge or respond to an insulin tolerance test) as surrogate predictors of late-life health and survival[3,8].

Linda Partridge: The markers to be used would depend on the process of disease progression, but they could include, for instance, markers of specific immune responses in trials of resistance to infection, and measures of musculoskeletal strength for osteoporosis and sarcopenia. Clinical trials should be focused on at-risk but disease-free cohorts, which are therefore likely to yield an adequate number of cases for powerful statistical analysis over the course of a short-term trial. Individuals at risk for developing specific age-related diseases could be identified by genotyping, if there are already known genetic associations, or by detecting the presence of early markers of disease risk, as in the case of atherosclerosis[9]. Individuals with lifestyle or environmental risk factors for specific conditions could also be targeted in early trials. Often there are large sex differences with respect to the risk of specific conditions, and thus to increase statistical power, at least initial trials could be focused on either females or males, depending which sex is at greater risk of developing the condition under study.

Jan van Deursen: With the intrinsically complicated heterogeneity of humans, establishing a molecular ‘marker profile’ that constitutes biological, not just chronological, age will be challenging if not impossible. We should therefore primarily focus on outcome measures that are relevant to quality of life, such as pain control and the ability to perform activities of daily living. I personally don’t think that we should fully sacrifice functional measures in exchange for the convenience of molecular biomarkers, which may only be relevant to aging in a subgroup of patients. Previous short-term Alzheimer’s disease clinical trials, for example, have been plagued by the use of surrogate markers that may show changes that are not reflected well in long-term functional outcomes[10–12]. We can avoid this trap at the outset by investing in medium-term trials on the order of a decade in length, which can accurately capture the effects of interventions during the last 10–15% of a lifespan. The use of biomarkers as surrogates for age-related functional decline should be ideally restricted to drug testing in middle age, before the window in which aging begins driving overt pathology or functional changes, and only used then because there is no alternative.

Saul Villeda

Saul Villeda: It is becoming appreciated that aging does not follow the same chronological trajectory in all individuals; rather, studies are beginning to indicate that signs of biological aging can even be observed in some individuals at young ages[13]. It may be that looking at markers associated with biological aging, even in young individuals, may prove to be key. For example, deficits in physical functioning such as motor ability or grip strength are relatively simple markers that have been reported to correlate with biological aging even in young individuals[13]. Along these lines, associations have also been reported between gait impairments and increased risk of age-related cognitive deficits in older adults[14]. Therefore, focusing on biomarkers of biological aging across age groups may provide a platform of tractable readouts that can be measured in a clinical trial without bias toward chronological age.

How do we model the complex influence of the environment on aging? How do we translate any related findings in model organisms to humans?

Rafael de Cabo: Over the past few decades there has been increasing research interest in studying interactions among genes, environment, nutrition, lifestyle and behavior. Multiple private and NIA/NIH-funded programs are looking into the complex interactions between environment and the aging process. For example, the Dog Aging Project, led by Matthew Kaeberlein and Daniel Promislow at the University of Washington in Seattle, is testing rapamycin in domesticated dogs[15]. The use of pet dogs is a unique design demonstrating an intervention in a heterogeneous animal population living in a human environment. This may be an ideal way to determine whether a drug such as rapamycin can affect aging characteristics and disease in an animal that lives, eats and often shares a bed with its owner. Human studies are finding that pathology and loss of function in old age have profound roots into the early events of life and lifetime environmental and behavioral exposures.

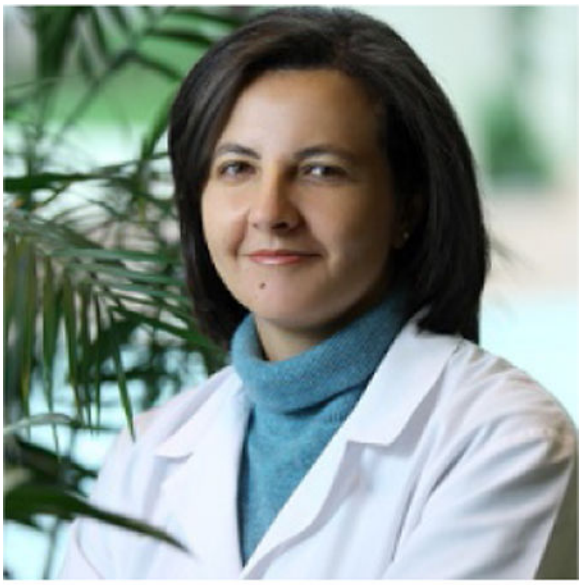
Longitudinal studies with a life-course design will be needed to better clarify the role of these influences.

Joan Mannick: It is difficult to model precisely the complex influence of environment on aging. However, it may be possible in preclinical studies to identify downstream cell-signaling nodes that mediate the effects of multiple environmental influences on aging. These common nodes could then be targeted for anti-aging drug development. In addition, because the modification of specific environmental factors such as diet and exercise has been shown to affect aging in model organisms, prospective randomized controlled clinical trials can determine whether the modification of these factors also affects aging in humans.

Linda Partridge: The importance of the environment for human aging can, to some extent, be understood by work with animals. Some interventions, such as dietary restriction, have proved to be effective in improving health during aging, often also extending lifespan in nearly all organisms tested, including rhesus monkeys. This kind of evolutionary conservation is at least strongly suggestive of the idea that some shared mechanisms will prove to be important in human aging. Indeed, trials that include a short-term reduction in food intake have shown beneficial health effects in older people[16]. However, some important aspects of environmental effects on health during aging in people can probably only be fully understood with work on humans themselves. For example, pathogens tend to be host specific and geographically regionalized. A better understanding of the effect of previous infections, and of exposure to other antigens, on age-related inflammation and autoimmunity is likely to require the study of the role of specific pathogens in populations that are normally exposed to them[17]. Psychosocial factors and stress have been shown to have important roles in human life expectancy, and here again the study of humans themselves is likely to be needed to unravel the mechanisms involved.

Jan van Deursen: Over the last several decades, improvements in animal husbandry practices such as water acidification and selection for long-lived mouse strains has led to an extremely high bar being set for testing anti-aging strategies in mice. Although detecting lifespan or healthspan improvements in long-lived mice is undeniably convincing, we must appreciate that not all human populations are on a trajectory to age under stress-free circumstances. Therefore, we are probably missing interventions that could mitigate stresses that are relevant drivers of aging in humans. This is illustrated well by the Interventions Testing Program (ITP) of the NIA, which did not detect lifespan-extending effects of rapamycin in long-lived mice at one test site, but did in two other, shorter-lived mouse cohorts. Although it is unlikely that we can deliberately ‘scale’ a mouse cohort to mimic life expectancy in the United States versus some theoretical human optimum, studies conducted under husbandry conditions with defined stressors should be performed and valued, rather than dismissed as suboptimal. This is not to say that we should surrender well-controlled scientific studies, but that we should introduce more, deliberately diverse stressors, such as sleep disruption, pathogen exposure, and poor diet, all of which are known to influence human health.

What is the value of targeting conserved pathways, and what role will combinatorial therapies have? Are our strategies likely to come from drug repurposing or should we focus on mimicking dietary restriction?



Mary Armanios

Mary Armanios: It is tempting to imagine that manipulating conserved aging pathways in combination, if it were possible, could tackle multiple diseases simultaneously. This view, however, contrasts with the emerging idea in precision medicine that effective therapies must be tailored to a given individual's biology for optimal benefit. The latter notion posits that each individual has unique personal values and genetic, epigenetic, and environmental factors that contribute to health. In this paradigm, a tailored approach that is informed by an individual's preferences, as well as by the relevant biological factors, would have the greatest impact. Examples over the past decades from molecular oncology indicate that an individualized approach can have the highest likelihood of changing the natural history of a disease process. With respect to aging biology, I will borrow an example from the short telomere syndromes. In people affected by this genetic diagnosis, stem cell failure causes degenerative disease in the bone marrow and lung[6]. Although these individuals have a premature aging disorder, they manifest only a subset of age-related disorders (one notable example is the absence of cardiovascular disease). A hypothetical combination therapy against multiple conserved pathways in these individuals may thus not be necessary and could theoretically result in adverse events. The approach of precision medicine is more compelling, but a major investment in multiple areas of research, both basic and clinical, will be necessary to fully realize its possibilities at the bedside.

Rafael de Cabo: Since the discovery in *Caenorhabditis elegans* of the first longevity-associated gene[18], *age-1*, conserved pathways have provided essential information about plausible targets that influence the onset and progression of aging and associated

morbidities. This relevance is further underscored by the high rate of success of the NIA ITP, which has identified positive hits (i.e., increase in survival in at least one sex of mice) in 5 out of 17 compounds tested. Compounds such as rapamycin, metformin, and resveratrol have been shown to prolong life and/or preserve health late in life in multiple model organisms, from yeast to monkeys[19]. Calorie restriction (CR), a 40% reduction in caloric intake, is the most powerful non-genetic intervention to delay the onset and progression of most chronic diseases and extend lifespan[17]. CR mimetics were conceived as an alternative to food deprivation, an intervention that is unlikely to appeal to humans[20]. Modeling drug candidates to mimic mechanisms of CR has proven to be a good strategy[21]. However, thus far, no single drug intervention has achieved the degree of health and lifespan extension that CR or single-gene manipulations have, but there are countless drug-gene interactions that are still untested; one of them may be the panacea for improving health and survival.

Joan Mannick: Demonstration that a specific pathway regulates aging across multiple strains of multiple species in multiple laboratories increases the likelihood that this aging pathway is also conserved in humans. Such conserved pathways are valuable targets for anti-aging drug development. Repurposing drugs that target conserved aging pathways is useful because the safety profiles of repurposed drugs often are well established. Knowledge of the safety profile of a drug is important for determining the aging-related conditions for which the drug is likely to have an acceptable risk/benefit ratio. It is probable that multiple pathway perturbations contribute to aging in a given individual. For instance, gene expression studies have revealed organ-specific pathway perturbations during aging[22]. Therefore, it is possible that combination therapy targeting more than one pathway will be of clinical benefit in aging-related conditions. It is also possible that anti-aging therapeutics will need to be personalized. For instance, 40% CR extends lifespan in some strains of mice but shortens lifespan in others[23]. Further elucidation of the precise nutrients and pathways mediating the lifespan and healthspan benefits of CR will be important for developing more targeted CR-based therapies for aging, and for identifying human subpopulations that may benefit from these therapies.

Linda Partridge: The major value of conserved pathways in aging is that we can study their mechanisms of action in short-lived animals, allowing detailed elucidation of molecular mechanisms, identification of drug targets, and pharmacological manipulation *in vivo*. Targets identified in this way are often already under intense scrutiny by the pharmaceutical industry in the context of specific diseases, such as cancer and metabolic disease, and thus drugs and chemical probes are readily available for testing. Some drugs are already proving to have a broader range of action than their on-license applications would suggest. For instance, rapamycin, an inhibitor of the kinase mTOR, is capable of extending lifespan in diverse organisms. Its main on-license applications are the prevention of restenosis after surgery and immunosuppression after tissue transplant. However, rapamycin has also been effective in boosting responses to vaccination[24] in humans and in protecting against neurodegenerative disease in animal models. Repurposing of existing drugs, especially those that can extend animal lifespans, is therefore likely to have an important role in targeting human age-related disease. More than one pathway can be targeted to improve health, and the mechanisms by which each pathway does so are, at least to some degree, independent

of each other. Therefore, pharmacological interventions that are likely to have maximum benefit in humans may consist of a polypill with multiple targets.

Jan van Deursen: Drug repurposing is useful from a safety standpoint, especially when considering theoretical treatment regimens lasting decades if aging is the outcome measure being tested. This safety-conscious motivation was one of the reasons metformin, which has been safely used for 60 years, was chosen for the first true healthspan test in the Targeting Aging with Metformin (TAME) trial. However, we should not restrict our imaginations with the safety concerns of lifelong interventions, as some useful strategies may require only intermittent application. For example, rapamycin improves the immune response to the influenza vaccine in the elderly when given during a short time window at a low dose, but long-term administration may be immunosuppressive. Additionally, short-term CR in early youth increases lifespan and produces lifetime benefits on metabolism and insulin sensitivity. This was shown in mice by Richard Miller's laboratory through crowded litter experiments in which pups were made to compete with added peers for nutrients during the first three weeks of life[25]. We should also consider that a combination of strategies might need to be applied to extend healthspan. We have to keep in mind that strategies may be counterproductive if applied at the wrong time. For example, CR appears to be useful in youth or middle age, but higher adiposity is protective in extreme old age. Similarly, senescent cell clearance may be useful throughout adult life but contraindicated during wound healing.

Saul Villeda: There is great value in targeting conserved pathways, but it may be necessary to consider combinatorial interventions that take into account how different pathways interact with one another. For example, there has been much excitement about studies in mice looking at the rejuvenating effect of young blood on aging phenotypes[26,27]. However, many of these same studies point to a parallel mechanism by which old blood can promote aging[26,27]. Each of these blood-centric mechanisms may provide a therapeutic target on its own, but the net additive effect of targeting both young and old blood may prove more robust than either therapeutic intervention alone. This same principle may be applicable across multiple systemic interventions, including CR. Furthermore, as more mechanistic insight is obtained from such laboratory studies, it is important that investigations into the potential synergistic effects of different mechanisms also accompany them. This line of research will also provide much-needed insight into the potential for deleterious combinations that should be avoided when moving forward toward human applications.

What is going to be the probable role of 'prescribing' lifestyle interventions versus pharmacological interventions?

Mary Armanios: There is ample evidence that lifestyle interventions promote healthy aging. Their implementation at the bedside requires concerted health policy efforts that prioritize prevention, education, and overcoming barriers of health disparity. These interventions, if fully implemented, can have an immediate impact on public health. Any potential pharmacologic interventions should therefore be tested head-to-head with lifestyle interventions.

Saul Villeda: The beneficial effects of lifestyle interventions such as exercise and CR are already known. However, the population as a whole is still not incorporating these into their daily routines. Many personal, cultural, socioeconomic, or other environmental factors weigh into the cost-versus-benefit of making these changes. The challenge we have as scientists is to understand how these lifestyle interventions work on a biological level, and then to provide the population at large with an alternative means by which to benefit from this knowledge. This is where pharmacological interventions will come into play. One aspect of this will be to identify novel factors that underlie the beneficial effects of interventions such as CR. However, it will be equally promising to investigate the beneficial—and potentially combinatorial—effects of repurposing drugs that have already been identified from previous studies involving these interventions in order to understand which pharmacological interventions yield the most-robust and safest results. We may not have to completely reinvent the wheel, but rather we need to understand which pieces that we already have fit best together.

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