AGS Geriatrics Healthcare Professionals

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Models and Studies of Aging: Report from the U13 Conference Series

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ABBREVIATIONS

AGS	American Geriatrics Society
CVD	cardiovascular disease
DMD	Duchenne muscular dystrophy
FDA	U.S. Food and Drug Administration
GEMSSTAR	Grants for Early Medical/Surgical Specialists Transition to Aging Research
IND	investigational new drug application
IRB	institutional review board
MCI	mild cognitive impairment
MI	myocardial infarction
NIA	National Institute on Aging
NIH	National Institutes of Health
PCT	pragmatic clinical trial
PI	principal investigator
PRECIS	Pragmatic-Explanatory Continuum Indicator Summary
TAME	Targeting Aging with Metformin

INTRODUCTION AND OVERVIEW

On September 21-23, 2016, the American Geriatrics Society (AGS) convened a conference in Bethesda, Maryland, to explore models and studies of aging. This was the second of three conferences, supported by a U13 grant from the National Institute on Aging (NIA), to aid Grants for Early Medical/Surgical Specialists Transition to Aging Research (GEMSSTAR) awardees in integrating geriatrics into their subspecialties. The conference brought 52 GEMSSTAR/Jahnigen/Williams scholars together with senior basic, clinical, and translational researchers and NIA staff. The conference participants represented a wide array of subspecialties. In addition to geriatrics and gerontology, the medical subspecialties represented at the conference included infectious diseases, obstetrics and gynecology, general internal medicine, cardiology and vascular medicine, gastroenterology, nephrology, anesthesiology, emergency medicine, and psychiatry. A total of 110 participants attended the conference. Drs. James Kirkland, of the Mayo Clinic, and Raymond Yung, of the University of Michigan, served as co-Chairs for the meeting.

In keeping with the overall topic of the conference, GEMSSTAR awardees also heard talks on the pillars of aging, the challenges of moving interventions from preclinical studies to phase III clinical trials, proceedings from a recent R24 activity, pragmatic trials, and NIA research directions. This report summarizes the scientific presentations of the meeting. The conference emphasized networking and mentoring. GEMSSTAR awardees participated in small-group sessions to refine their research pitches; engaged in a networking session with program officers, mentors, and scholars from the National Institutes of Health (NIH); presented their work during a poster session; and heard talks from senior investigators and NIH staff on aspects of career development and resources for junior investigators.

PILLARS OF BIOLOGICAL AGING AND IMPLICATIONS FOR THE SPECIALIST

Overview

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The phenotypes that emerge with aging are very complex and extremely variable among individuals. In spite of such complexity, it is possible to classify these phenotypes into four domains: neurodegeneration, homeostatic dysregulation, energy imbalance, and changes in body composition. It has been hypothesized that changes in these phenotypes is driven by interconnected biological mechanisms that are intrinsically connected with aging and have been called the hallmarks or pillars of aging. For example, several studies of aging-related gene expression have shown alterations for genes associated with inflammation. High inflammation, as defined by high levels of IL-6 in the blood, is a powerful predictor of multimorbidity, both cross-sectionally and longitudinally. Yet recent proteomic work in CD4+ T lymphocytes has shown that almost half the alterations observed from older animals occurred in mitochondrial proteins associated with electron transport chain complexes. Despite downregulation of the overall mitochondrial fraction of gene expression, and despite reduced mitochondrial respiration capacity, mitochondrial proteins associated with electron transport chain complexes are upregulated in CD4+ T lymphocytes from older animals. Moreover, even though the number of mitochondria does not appear to change between younger and older adults, autophagy is more defective among older mitochondria. Thus, the altered intercellular communication underlying inflammation is connected with mitochondrial dysfunction and changes in proteostasis.

Changes in energetics constitute another hallmark of aging. Mitochondrial function, as measured by phosphocreatine recovery following exercise, has been associated with walking speed, a more traditional measure of health in older adults. This association is explained in part by the effects of mitochondria on the ability of the muscle to contract during lower-extremity performance. A recent study has found that mitochondria reside not as discrete organelles, but as connected networks, with energy generated in a subset of the mitochondria and diffusing toward the center of the network. This same study also has demonstrated that aging is associated with an accumulation of fat that severs the connections between the different parts of the mitochondrial network, thereby fragmenting communication and interrupting the intracellular diffusion of energy. Pre-clinical evidence suggests that connections are lost at a lower rate among animals that exercise, compared with those that do not.

Other hallmarks of aging include epigenetic alterations, cellular senescence, loss of proteostasis, and genomic instability. Evidence suggests that, in some tissues, age as characterized by DNA methylation tracks with chronologic age. The association between age and accumulation of senescent cells is well established, and accumulation of senescent cells expressing $p16^{INK4A}$ appears to shorten health span. Emerging evidence also suggests

that senescent cell accumulation, which can be measured both in the skin and in the peripheral blood, contributes to the pro-inflammatory phenotype seen with advancing age. Further evidence suggests that age-associated cellular senescence, which is marked by the inability to replicate and perform repair, arises from defective autophagy and an accumulation of unrecycled proteins. Age-associated accumulation of DNA damage and defects in DNA repair also suggest genomic stability as the first hallmark of aging. However, it is not yet clear how these problems influence aging, and the cells themselves are heterogeneous in the extent to which they are capable of repair.

It is likely that frailty arises from the failure of all these mechanisms to sustain health. However, the traditional view of normal or accelerated aging as a continuous, homogeneous curve is likely wrong. Aging more likely occurs at different rates across different tissues and across different individuals. Thus, the approach of applying the same intervention across a population would probably be ineffective. Emerging evidence from cohort studies are suggesting the need for intervention at earlier time points, but the best time for evaluation and effective intervention remains unclear.

Inflammation

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With age, multifactorial, low-grade activation of the innate immune system leads to the chronic production of inflammatory mediators. Whereas acute inflammation in response to infection is characterized by an initial but transient bump in IL-6 and C-reactive protein (CRP) expression, neutrophil recruitment, and the production of other acute inflammatory mediators, chronic inflammation is characterized by consistently elevated IL-6 and CRP, angiogenesis, mononuclear cell infiltrates, and fibrosis. Chronic inflammation involves the activation of NF-κB, a nuclear receptor that acts to induce the production of several inflammatory mediators in response to stimulation from TNF-α Toll-like receptor proteins (TLRs), free radicals, and other pro-inflammatory stimulants.

Chronic inflammation can be measured in humans via serum inflammatory cytokines. A study of the relationship between 15 cytokines and 10-year mortality demonstrated IL-6, CRP, TNF-α receptor 1 (TNF-α R1), IL-1RA, and IL-18 as the best serum inflammatory predictors of vulnerability.[1] IL-6 and TNF-α R1 individually or combined into an inflammation index best predicted mortality, walking speed, and deficiency in some cognition markers.[1-3] IL-6 has been associated with decreased performance and strength[3]; with the acceleration of chronic disease; with mortality[4]; and with multisystem dysregulation such as anemia, activation of the hypothalamic-pituitary-adrenal (HPA) axis, and declines in appetite and body mass.[5] Chronic IL-6 signaling also likely contributes to declines in muscle satellite and blood stem cells, the senescent cell proliferation that remodels the immune system, and mitochondrial dysfunction and fibrotic tissue changes. Chronic TNF-α R1 production contributes to apoptosis, necroptosis, and accelerated cell loss and drives further inflammatory activation and disease.

There is no simple or single cause that triggers and sustains chronic inflammation. Senescent cells, increased fat mass, damaged

mitochondria, alterations in the microbiome, and dietary and lifestyle factors all have been implicated. Likewise, chronic inflammation can trigger other potentially damaging processes via several mechanisms. Emerging evidence suggests that key stress response systems, including the renin-angiotensin system, the HPA axis, and the sympathetic nervous system, mediate the contribution of inflammation to vulnerability.

A model pathway to frailty and adverse outcomes suggests that aging hallmarks such as mitochondrial decline, changes in DNA methylation, and altered autophagy induce physiologic changes such as inflammation, which in turn contribute to clinically apparent phenotypes.[6] At present, exercise and good nutrition have been established as beneficial interventions against inflammation. In the future, however, the development of diagnostic measures and interventions will depend on the identification of the most important pathways, specific targets, and the best ways to modulate them. Angiotensin receptor blockers such as losartan have been successful in improving skeletal muscle in mouse models and are presently under study in humans.[7]

Metabolism

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Aging is associated with several metabolic changes, including diabetes, which is a major driver of vascular complications. The prevalence of impaired glucose tolerance and both diagnosed and undiagnosed diabetes increases with age,[8] approximately half of adults older than 65 have diabetes or pre-diabetes, and the incidence of major diabetes complications increases with older age.[9] Older age is associated with higher blood glucose concentrations and declining insulin sensitivity,[10] but the reason for these changes is unclear. Likewise, older age is associated with increasing intestinal barrier dysfunction,[11] but the reason for this is also unclear.

One model suggests that NF-κB serves not only as a gateway for chronic inflammation, but also as a central player in metabolic signaling. Stimulated by fatty acids, lipids, and mitochondrial dysfunction, NF-κB regulates pathways associated with ribosome synthesis, ubiquitination and proteasome function, and glucose metabolism. Older age is associated with higher lipid content, even among healthy individuals.[10] It is also associated with declines in mitochondrial function, resulting in reduced ATP production and a declining ability to oxidize lipids. Age-associated declines in mitochondrial function might arise in part from changes in molecular pathways governing mitochondria and the renin-angiotensin system: age has been associated with downregulation of PGC-1α and one of its key regulators, AMPK.[12] What drives the age-associated declines in bioenergetics is unknown.

Thus, how to treat older adults with diabetes is poorly understood. The American Diabetes Association and AGS recommend that treatment goals account for functional status, life expectancy, cognitive function, and clinical heterogeneity in terms of risk for complications. Only 20% to 40% of patients with diabetes develop microvascular

complications, and on average, it takes 15 years for those complications to develop. The risk for microvascular complications increases twofold[13] at hemoglobin A1C levels of 6% or 7%—the current treatment target is 7%—but how important hemoglobin A1C is to risk remains a point of debate. Many drugs, other than metformin and insulin, are now available for diabetes treatment, but it is not clear which of these drugs will serve as the next major advance. Further research is also needed to understand more fully the epidemiology, etiology, screening, diagnosis, preventive strategies, and complications of diabetes.[9]

Resilience and Adaptation to Stress

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Resiliency has been defined by Merriam-Webster as "the ability to become strong, healthy, or successful again after something bad happens" or "the ability of something to return to its original shape after it has been pulled, stretched, pressed, bent, etc." The term has long been of interest in mental and behavioral health, reflecting the ability of individuals to adapt well in the face of adversity, trauma, tragedy, threats, or emotional stress. However, the concept of physical and physiological resiliency was described as early as the 19th century, when Claude Bernard described the "constancy of the milieu intérieur," which describes the ability of an organism to maintain such constancy as external variations are instantly compensated for and equilibrated.[14]

The phenomenon of homeostasis and how it might change with older age was also described in the first half of the 20th century, when Walter Bradford Cannon noted that physiological investigation of old age might reveal "increasing limitation of the effectiveness of homeostatic devices which keep the bodily conditions stable." Homeostenosis refers to the diminished capacity of an organism to respond to various homeostatic stressors. Studies have shown that responses to stressors such as glucose challenge and changes in ambient temperature are not only less effective in old age, but might also take longer to return back to a baseline state.[14-17] Thus, diminished resilience as well as enhanced basal activity and loss of physiologic reserve are key features of age-associated homeostatic dysregulation. Other features include lower end-organ responsiveness and loss of negative feedback inhibition.[14,18]

Although many of these features are shared across different stressors and systems, resilience and adaptation to stress ultimately depend on the magnitude of the stressor, exposure to other preceding or coexisting stressors, and physiological responses to the stressor.[19] For example, a study by Carter et al. showed the importance of the magnitude of the stressor when demonstrating the effects of blood pressure medications on systolic blood pressure as a function of daily dose.[20] Another study showed no differences in blood pressure between older and younger individuals after they had been tilted.[21] However, when study participants were given a small diuretic dose prior to the tilt (stressor 1), blood pressure declined among older adults in response to the tilt (stressor 2).

Resilience in aging is not simply the absence or converse of frailty.[19] It is complex and multifactorial. No cell, organ, or system works in

isolation from others; complex interrelationships among all these entities are mediated by shared molecules, proximal risk factors, and distal outcomes with bidirectional feedback loops. Larger patterns might arise through interactions among smaller or simpler entities that themselves might not show such properties. Thus, a systems-based approach is critical in understanding and predicting resilience in older age. In addition, contrary to the paradigm of an overall aging trajectory in which an organism reaches perfection then declines, aging is a highly variable and heterogeneous process. Thus, this variability between individuals, which increases with age, must also be addressed, and not just at baseline, but even more importantly after individuals are challenged. Above all, complexity and variability observed among older adults should be embraced, as it is the variability that is most likely to offer novel insights and predictive capabilities with respect to resilient responses and clinical outcomes.

Biological Aging: Discussion

The clustering of chronic diseases is apparent not only in later life, but also with obesity, suggesting obesity as a model for accelerated aging. Although obesity is a risk factor for multiple diseases, however, its relationship with aging is complex. For example, some evidence suggests that losing weight can be disastrous late in life. In addition, although obesity is associated with inflammation, this association appears to be distinct between older age and inflammation. Aging is associated with increased production of inflammatory markers even among individuals with no risk factors and a healthy body mass index, and although exercise can reduce adiposity and is associated with a decline in inflammatory markers, inflammation is never fully resolved. Liposuction appears to have no effect on inflammation, whereas gastric bypass surgery is associated with a rapid decline in inflammation before weight loss becomes apparent.

Acute illness and admission to critical care are particularly detrimental for older adults. However, more research is needed to understand the transition between acute and chronic inflammation and how that transition might be affected by older age. Some work has shown that peripheral blood mononuclear cells from frail individuals have higher baseline levels of inflammatory mediators and are more likely to produce these mediators in response to lipopolysaccharide challenge. The inflammatory response to acute conditions such as pneumonia is similar between older and younger adults, but the resolution of the response, for example the normalization of inflammatory mediators, appears to be delayed among older adults.

Understanding of resilience in aging has been limited by the tendency of specialties to work in silos. For example, exercise appears to be an important factor across all the hallmarks of aging and particularly with respect to frailty and resilience, but the type or timing of exercise that would be most beneficial is not clear. Likewise, the clinical relevance of resilience remains poorly understood. More collaborative, integrative research is needed. Increased understanding and acceleration of that understanding into clinical practice can arise from broad collaborations among disciplines and the inclusion of clinical specialists in research teams.

INTERVENTIONS: MOVING FROM PRECLINICAL TO PHASE III STUDIES

Translation from Bench to Bedside

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The translation of a discovery from the bench to the bedside rarely follows a linear pattern. Instead, it often follows a winding path with unexpected turns. The Rando laboratory's path from muscular dystrophy research to potential therapeutics for Alzheimer's disease serves as an example. When Dr. Rando first became interested in Duchenne muscular dystrophy (DMD), he and his colleagues intended to study oxygen metabolism, based on compelling data suggesting that some characteristics of DMD were associated with free radical injury. At the same time, the laboratory began to investigate the response of the tissue to injury, namely stem cell-mediated tissue repair.

The model of tissue regeneration also provided a new avenue for DMD therapeutics. The rationale for stem cell therapy to stimulate regeneration in skeletal muscle was demonstrated in a 1977 publication.[22] A later study showed that a type of stem cell in muscle, a so-called "mesangioblast," could improve muscle function in dystrophic dogs.[23] This ultimately led to a phase I clinical trial of mesangioblast therapy in boys with DMD.[24] Nevertheless, it was clear that a better understanding of the biology of muscle stem cells would be necessary to develop this therapeutic approach. Thus, the Rando laboratory pursued studies in this direction, yielding publications, ranging from basic biology to translational studies,[25-31] including one demonstrating the role of Notch signaling in regulating muscle stem cell fate and function.[32]

Along the way, the Rando laboratory also became interested in the biology of aging after observing that Notch signaling could restore tissue healing in older mice at a rate similar to that seen in younger mice.[33] Pioneering use of the technique of heterochronic parabiosis to study cell and tissue aging, the laboratory showed that exposure of older muscle to a youthful environment rejuvenates aged muscle stem cells.[34] Further work identified protein and signaling pathways, including CCL11/eotaxin, Wnt, TGF-β, and β2-microglobulin, as mediators of cellular aging or rejuvenation[35-38] Colleagues of Dr. Rando also demonstrated that plasma from young mice can enhance cognitive function in older mice.[39] The company Alkahest is extending this model into humans, assessing the safety of plasma from young donors and the effects of this plasma on behavioral tests among patients with early Alzheimer's disease.

Although this story does not represent a classical path for drug development, it does illustrate that pursuing scientific interests can lead to findings that have the potential to be translated to therapeutic studies in humans. As investigators pursue the translation of bench discoveries, they should be willing to follow their science, play to their strengths, and collaborate to address their "lesser strengths."

Phase I and II

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The primary premise of geroscience supposes that targeting fundamental aging processes, which appear to form the largest risk factor for multiple diseases, will aid in delaying, preventing, or treating these diseases. Several interventions that target different aging mechanisms are making their way from basic biology studies in animals through early translation studies. Among these are senolytics, which clear senescent cells by targeting the survival pathways by which senescent cells resist apoptosis.[40] Studies in aged or progeroid mice have shown that senolytics delay neurologic dysfunction, enhance cardiac and vascular function, alleviate glucose intolerance, and alleviate radiation-induced gait disturbance. Pharmacologic or genetic clearance of senescent cells could be beneficial for more than 30 indications.

Senolytics and other exciting new agents targeting fundamental aging mechanisms could become beneficial clinical interventions if preclinical studies can demonstrate effectiveness and low toxicity and if clinical trials are done in a manner realistic for translation into human application. Animal models used for preclinical testing must be translationally relevant and acceptable to the U.S. Food and Drug Administration (FDA) for registration studies. Therefore, they must differ from models used for discovery research. For interventions targeting aging processes or age-associated diseases, preclinical models should include aged or frail animals, those fed high-fat diets, models of resilience, and other models exposed to clinically relevant insults. Likewise, toxicity testing requires age-appropriate models and should address short- and long-term toxicity and be acceptable for the FDA for inclusion in Investigational New Drug applications (INDs).

When moving interventions from preclinical testing to clinical trials, investigators should test effects on measurable, clinically relevant outcomes that are appropriate for older populations. The benefit of interventions for the study population needs to outweigh risk. Short-term studies need to be designed to include patient populations with compelling clinical need and the presence of multiple comorbidities. Clinical trials require biomarkers that are impacted by the intervention, correlate with relevant clinical outcomes, and change as those outcomes change. Studies should be designed to measure dosing and pharmacokinetic biomarkers in the blood and at FDA standards. Studies of agents targeting aging processes should assess effects on panels of mechanistic biomarkers related to the hallmarks of aging. Surrogate endpoint biomarkers that can serve as FDA-recognized indications, such as blood pressure as a surrogate for stroke prevention or LDL cholesterol for atherosclerosis, are unlikely to be acceptable to the FDA for aging studies in the near future.

Although phase I studies typically involve dose-ranging and assessments for safety in healthy (and primarily younger) volunteers, trials testing agents that target aging processes might be served better by enrolling older participants, particularly those with multiple comorbidities. Phase II studies, which assess safety and efficacy, tend to enroll substantially more patients,

compared with phase I trials. It should be noted that efficacy, or whether an agent provided in the specific manner described by the study, differs from effectiveness, or whether a treatment will influence a disease when patients are treated as they would be in the real world.

Even with a natural product, interventional studies in humans require an IND approval from the FDA and several levels of approval and oversight. INDs can be time- and labor-intensive, requiring information about preclinical effectiveness, safety, manufacturing, toxicology, and trial protocols, among other things. Clinical trialists could therefore benefit from the establishment of infrastructure that would assist them in preparing INDs. Clinical trials must be approved by institutional review boards (IRBs). In general, clinical trials could provide basic biology laboratories with human tissues across the age range and before and after interventions. Studies of agents that specifically target basic aging processes could use several clinical scenarios, such as simultaneous alleviation of comorbidities, accelerated aging conditions, conditions involving localized cellular senescence, resilience, clinical stresses in pre-frail individuals, or frailty.

Phase III

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As discussed throughout the conference, aging is the strongest risk factor for all age-related diseases,[41] and the evidence suggests shared mechanisms across these diseases. In addition, data from the Rochester Epidemiology Project indicate that the incidence of having more than three comorbidities is skyrocketing.[42] Thus, even if one disease is treated, patients are likely to develop another because the rate of aging has not been addressed.

The potential benefit of delaying aging has been suggested by the extension of healthy lifespan in several animal models and by certain benefits observed with relevant drugs such as metformin. As demonstrated by the Diabetes Prevention Project, metformin delays the onset of type 2 diabetes and, in patients with diabetes, it delays cardiovascular disease (CVD). However, it is also associated with less cancer in patients with type 2 diabetes, delays aging in nematodes and mice, and appears to target several pathways associated with aging.[43] Evidence also suggests that treatment with metformin can lower the incidence of multimorbidity.[42] Recent studies have shown that patients on metformin are at lower risk for neurodegeneration, dementia, and Parkinson's disease,[44] and that patients with mild cognitive impairment (MCI) show significant changes in cognitive parameters after taking metformin for 1 year.[45] A phase IV-like case-control study in the United Kingdom has found that metformin decreases mortality in individuals with or without diabetes, even among obese patients, compared with sulphonylurea monotherapy.[46]

Phase III clinical trials build on effectiveness and safety data generated from phase I and II trials and aim to obtain additional evidence to inform the evaluation of overall benefit and risk and to provide an adequate basis for physician labeling. They are driven by outcomes and often compare new agents with currently used interventions. However, real-world data on efficacy, effectiveness, adverse events, optimal use, and comparative use are generated only in

phase IV trials, which are conducted following FDA approval and initial clinical use of an agent. In addition, indications for age-related comorbidities and aging itself have not been defined by the FDA to guide testing in phase III trials.

The Targeting Aging with Metformin study (TAME) aims to demonstrate that metformin can target age-related multimorbidities and aid the FDA in developing a practical definition of aging as an indication based on multimorbidities.

TAME will evaluate the time to new treatment for a composite endpoint comprising CVD, cancer, MCI or dementia, and death among 3,000 patients stratified into individuals at high risk for these diseases and those with a history of at least one of these diseases. Secondary endpoints include aging phenotypes and time to onset of diabetes. If successful, TAME can provide a paradigm for studying next-generation drugs targeting multimorbidities and for applying discoveries from geroscience in primary prevention of multiple diseases.

PRAGMATIC TRIALS

Overview

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The NIH spends approximately $3 billion on clinical trials.[47] However, the majority of studies registered on ClinicalTrials.gov are small and heterogeneous in terms of methods.[48] Many unanswered questions remain, for example, only 15% to 30% of cardiology guidelines are based on the highest-quality evidence.[49] Other approaches are needed to tackle treatment of multiple chronic conditions, which constitute the most common chronic condition among older adults.[50] Such approaches are also needed to support clinical and Medicare decision-making.[51] By law, Medicare is required to base coverage decisions on whether an intervention is reasonable and necessary. However, "reasonable and necessary" is not well specified, and despite efforts to clarify the phrase in practice, there is still not enough evidence for many decisions, and some coverage decisions have relied on low-quality evidence. Clearly more real-world evidence is needed.

Pragmatic clinical trials (PCTs) are designed to inform health care decision-makers, which can include patients, clinicians, and policymakers. They compare clinically relevant alternatives, enroll diverse study populations from the real world, recruit from several practice settings, and measure a broad range of relevant health outcomes or streamline data collection to ensure adequate power.[52] PCTs differ from explanatory trials by offering broader eligibility, flexible interventions, typical practitioners, no follow-up visits, and usual adherence.[53] However, these differences are not absolute. The Pragmatic-Continuum Indicator Summary (PRECIS) Spokes model[53] has been developed to assess how pragmatic or explanatory a trial

is. FDA Commissioner Robert M. Califf, MD, has called for pragmatism in all clinical trials.

The number of PCTs has been growing since the 1990s. One PCT, the MIFREE study, has explored full coverage for preventive drugs versus usual care (paying a copay) following a myocardial infarction (MI) and found that enhanced coverage improves some outcomes, with neutral overall costs.[54] These results led Aetna, the trial sponsor, to cover preventive drugs for all MI beneficiaries. Another PCT has found that mild nudges such as accountable justification, peer comparison, and suggested alternatives can curb inappropriate antibiotic prescribing among primary care practices treating patients for acute respiratory infections.[55] Yet another, PROVEN, is assessing the use of short videos on initiating advanced care planning among nursing home patients with advanced comorbidities. Although Medicare requires nursing homes to offer advanced care planning, and despite evidence that these videos improve outcomes, such videos are underused. PROVEN is comparing outcomes among nursing homes that integrate these videos into their operations with those in control facilities.

In 2016, the NIH offered three funding opportunity announcements supporting PCTs. One, funded primarily by the National Institute of Diabetes and Digestive and Kidney Diseases, supports pragmatic research in health care settings to improve prevention and care for obesity and diabetes. Another, supported by the National Institute on Mental Health, supports pilot effectiveness trials for treatment, preventive, and services intervention. The third, supported by the NIA, supports pragmatic clinical studies to evaluate patient-centered outcomes. In addition, a funding announcement from the NIA is bridging the R21 and R33 mechanisms to support studies that use behavioral economics to encourage appropriate care. The NIH is expanding the use of PCTs to study effectiveness of late-stage dissemination of novel health care delivery approaches.

Finding Research Questions

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Many ideas that are now part of mainstream medicine, such as physical resistance training for people recovering from major physical trauma (Pilates), relaxation and breathing techniques to ease the pain of childbirth (Lamaze), the benefits of breastfeeding for infants (Froelich), and the need for extensive palliative support and reduced medical intervention for dying patients (Saunders, Kubler-Ross), were resisted at first. On the other hand, some practices established as beneficial appear to be less so when applied to certain subpopulations, such as frail older adults. In addition, there are several examples in which the medical profession did not arrive at the right answer. Despite a dramatic rise in the rate of early-stage cancers diagnosed, widespread mammography screening has had no effect on late-stage cancers.[56] Although sleep apnea has been associated with several adverse health outcomes, including MI, a recent study has found identical rates of cumulative cardiovascular events between patients who receive continuous positive airway pressure therapy and those who do not.[57] Despite the conventional wisdom that infants should

consume only breast milk to avoid allergies, more recent evidence suggests that early exposures to allergens can reduce some forms of allergies.[58]

There is no single cause for wrong or incomplete answers. Instead, such answers can arise from a variety of factors, including a lack of fundamental understanding of the underlying biology, inadequate research tools or inappropriate outcome measures, a lack of unbiased efficacy data, or limitations of such data, including a lack of external validity or heterogeneity in treatment effect. Many important questions that are not answered by explanatory clinical trials are those that question the conventional wisdom. Such questions most likely will be answered with pragmatic, real-world evidence.

The majority of clinical studies focus primarily on efficacy, but they do not necessarily have to start there. When considering potential research questions, investigators can consider all aspects of the spectrum from basic science (how does it work), to translation (how can we study it in people), efficacy (is it beneficial), and effectiveness (how effective is it in the real world). To begin to question the conventional wisdom, investigators must understand the potential intervention, what the clinical alternatives are, and, particularly, what doing nothing means. They must understand the measures of effectiveness, how they behave, and how to refine them.

It should be noted also that pragmatism is not mutually exclusive of improved precision. Indeed, pragmatic trials and precision medicine can be complementary in exploring risk and benefit. The application of the conventional wisdom across a large population might mask differences. For example, although all participants in an interventional study might be at risk for a particular adverse event, that risk will vary broadly.[59,60] If the benefit of an intervention is limited to participants with the highest risk, the intervention could be beneficial only to a small subgroup within the study but present harm to a substantial proportion of participants who are at lower risk. Thus, means and averages can be misleading. The potential heterogeneity of a treatment effect underscores the need for large-enough sample sizes and datasets to perform risk stratification and conduct subgroup analyses. Investigators therefore must understand how to quantify research questions and how to approach data statistically.

The NIH Health Systems Collaboratory, a Common Fund project, is funding 10 pragmatic trials that are similar to PROVEN in scope. In each trial, an investigative team works with a health care systems partner such as Kaiser Permanente or the Nursing Home Network; the trials use a variety of approaches to work with these systems. All 10 trials examine care in real-world settings. Most have a cluster randomization design and use methods to extract answers from electronic health record data. Although the studies aim to disrupt processes as minimally as possible, they are also assessing how best to implement clinical practice guidelines. With careful attention to the protection of human research participants, both the study sections and IRBs associated with the Collaboratory have become comfortable with pragmatic approaches.

PROCEEDINGS FROM THE R24

Overview

Aging research has moved from a primary focus on descriptive, mechanistic research toward a focus on interventions. As discussed during the first day of this conference, the geroscience hypothesis suggests that targeting fundamental aging processes will delay, prevent, alleviate, or reverse multiple geriatric syndromes, chronic diseases, and loss of resilience. At present, geriatric medicine focuses on tertiary prevention, complications of chronic diseases, geriatric syndromes, and aids and devices such as walkers and wheelchairs. The prevailing view is that interventions targeting fundamental aging processes will be useful only if they are administered before the beginning of disability. In 10 years, however, advances in the biology of aging could lead to interventions that compress morbidity and delay chronic diseases and geriatric syndromes. In addition, interventions could also be beneficial to older individuals with multiple morbidities.

The Geroscience Network, supported by an R24 from the NIA, comprises 18 institutions focused on the geroscience hypothesis and the approaches needed to answer these questions. Involved in the Network are institutions in the European Union, which are separately funded, and MouseAGE, a consortium of 22 institutions focused on developing aging mouse models. The Network has held seven retreats and published six papers on R24 progress in a special issue of the Journal of Gerontology Series A. These activities are closely aligned with two workshops the NIA has held on resilience in aging animal models and physical and physiological resilience in humans.

BARRIERS TO THE PRE-CLINICAL DEVELOPMENT OF THERAPEUTICS THAT TARGET AGING MECHANISMS

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Mayo Clinic

The first retreat, held in 2014 at the Scripps Research Institute in Jupiter, Florida, explored barriers to the preclinical development of therapeutics that target aging mechanisms.[61] Although retreat participants began with strong but varied perceptions about the ideal preclinical pipeline, they were able to develop consensus.

Among the five discussion topics were funding and the integration between researchers and clinicians. Researchers agreed that funding should not be taken away from descriptive and mechanistic research, as these types of studies generate new discoveries that prime the drug development pipeline. The NIA has worked with other NIH Institutes and Centers to highlight the importance of aging to their respective research missions. Retreat participants also discussed

difficulties translational proposals face in study sections. Study section members are less likely to discuss and score proposals outside of their own areas of expertise, and points of view—for example, with respect to study sample size, outcomes of interest, or design of statistical analyses—differ between clinicians and basic scientists. In addition, applications that include both basic and clinical research cannot devote sufficient attention to both areas within the page limitations allowed for grant applications, yet they must compete with applications that are devoted entirely to either basic or clinical science. Retreat participants suggested that these problems be addressed by splitting grants into their basic and clinical components or by establishing special study sections.

Infrastructure to support early phase forward and reverse translation was also discussed. Researchers noted difficulties in accessing biobanks and obtaining tissue samples. They called for the creation of a national experimental and clinical biobank in the United States, as well as for tissue-sharing agreements among institutions. Researchers also agreed on the need for a central core or facility to assist with preparing INDs and called for the development of shared protocols, standard operating procedures, and coordination to ensure that preclinical and translational studies make appropriate comparisons. A related concern was competition among researchers and commercial entities, which increases as fields move toward translation. The move away from collaboration and openness to competition and secrecy interferes with collaborative, multicenter academic clinical trials and with the sharing of important information about potential new interventions.

A major point of discussion at the retreat was the need for personnel with a sufficient grasp of aging biology, IND clinical trial design, and geriatrics. Retreat participants recommended training for geriatricians in basic biology and for basic biologists in translation. A transnational network of aging centers is under development. It would be helpful to develop formal links with the National Center for Advancing Translational Sciences and with European Union networks. Retreat participants have called for the development of a national preclinical studies network as a follow-on from the R24 initiative. Immediate next steps include small-scale trials, infrastructure development among institutions that want to collaborate, and training programs for basic scientists and clinicians to develop personnel with expertise in translational studies.

Evaluating Healthspan in Pre-Clinical Models of Aging and Disease: Guidelines, Challenges, and Opportunities for Geroscience

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Caloric restriction has emerged as one of the strongest models for aging research and for extending the human lifespan.[62] Ongoing work has identified several molecular pathways,[63] as well as compounds, such as resveratrol, that can improve health and survival. Studies also have shown gender- and context-specific effects. For example, rapamycin is more successful in extending longevity in female mice,[64] whereas acarbose is more successful in males.[65] To promote successful aging, work is now needed to understand the basic mechanisms underlying the responses to these interventions and the phenotypes that are altered by them.[66,67]

Among humans, there is tremendous heterogeneity both in aging and in response to medications. Work in mice shows similar heterogeneity, even in a genetically homogeneous population. Tests of gait speed and frailty have shown predictability in humans[68-70] and in cross-sectional studies of mice. Now these tests are being used to assess potential variations in trajectories in a study of longitudinal aging in mice. This study is collecting data at different time points on several parameters in two strains of mice. Some parameters, such as glucose or lactate concentration and grip strength, have shown variation at baseline, and the study will assess whether these variations are predictive as the mice age. The study is also assessing metabolic rates and magnetic resonance imaging. This study ultimately aims to identify markers for aging-related outcomes that will predict good health and survival and can be used to create and validate interventions.

Strategies and Challenges in Clinical Trials Targeting Human Aging

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Two recent workshops have explored strategies, challenges, and frameworks for proof-of-concept and clinical trials of interventions derived from studies in aging biology. Four themes emerged from these discussions:

Target big picture outcomes. Not only is aging the primary non-modifiable risk factor for several chronic diseases, it is also the prism or processor that determines how diseases manifest in people's lives. Thus, clinical studies in aging should focus on integrative, big-picture outcomes that are important to people's lives, such as functional outcomes and independence, geriatric syndromes, and mortality. The workshops recommended extending the health span as one framework, where targeting the mechanisms underlying aging could prevent or delay the losses and declines associated with aging, and studies would assess global outcomes such as multimorbidity or daily function. Enhancing resilience was also recommended as a framework. At every level, from the cellular and molecular level to the whole person, aging presents universally as a weakened ability to cope with new stresses and stimuli. Within this framework, interventions that target the mechanisms of aging could reduce the period of decline or enhance the period of recovery following stress, again at various levels.

Study subjects. The majority of clinical studies still look for ideal participants, for example patients with no comorbidities. Although there is some mechanistic value to studying interventions in the ideal population, these agents also should be studied in populations that will actually be treated. Clinical trials should include older people, particularly those who are frail or have multiple morbidities, and embrace heterogeneity. The choice of study population will depend on the intervention under study, the presence and severity of illness and how that affects outcomes of interest, and the window of opportunity for intervention. Small pilot studies should be conducted to determine the best time and approach for applying interventions. In addition, clinical studies should be considered within the overall context of aging: how is the disease of interest affected by the hallmarks of aging, their biochemical markers, and the

interactions among them; how is the disease of interest influenced by other chronic diseases, and what markers should be used to measure them, and which global outcomes should be measured?

New tools for helping older adults. Studies in aging biology could yield new interventional tools that clinicians can use to affect their disease of interest. Among potential interventions emerging from basic and clinical studies are those, such as metformin, acarbose, angiotensin receptor blockers, and angiotensin-converting enzyme inhibitors, that are already approved by the FDA but could play broader roles in aging. However, many agents have not been tested specifically in frail or older adults. Existing clinical programs targeting older adults can be leveraged to provide infrastructure and comparisons in clinical studies. These tools and clinical studies can also contribute to an evidence base to support aging as an indication on which the FDA could base approvals of interventions. Composites of existing outcomes, for example multiple diseases, could also be used.

Accelerating translation. Workshop discussions also focused on how to facilitate the incorporation of aging into clinical studies. Suggestions included the development of cognitive and physical infrastructure to accelerate research; subspecialists' integration of aging concepts into their studies; a shared library of templates for trial designs, INDs, IRB proposals, and data safety monitoring board reports adapted to older adults and aging-related outcomes; a standardized, modular outcome toolkit; and a national geroscience biobank that is diverse and enriched for multimorbidity and frailty.

Discussion

Whereas only one intervention to extend lifespan was available from 2000 to 2004, there are now 40 new potential interventions that might be context specific. Work is underway to identify signaling molecules that are best targeted for specific diseases in specific contexts. The NIA is supporting efforts to identify blood markers that can predict functional declines and differential responses to aging, with the goal of aiding in drug selection. Investigators hope that within 5 to 10 years they will be able to identify which biomarkers should be tested and how and when to test them. At the same time, more work is needed to understand resilience across the life course. For example, increased understanding of how one gains resilience in childhood could give rise to interventions, such as dietary changes, that optimize what patients already have. Studies in preclinical models are already exploring how early one should intervene to provide the best benefit. However, lifestyle interventions, while important, take a long time to gain traction. In addition, antagonistic pleiotropy, where drugs that are beneficial in younger people prove to be harmful in older adults (or vice versa), should be addressed. A multi-pronged approach to aging might be best.

Although the geroscience hypothesis offers a universalist perspective to chronic disease, aging is also variable across individuals. In addition, health disparities remain a problem. Environmental factors such as socioeconomic status and social networks predict health outcomes and have been demonstrated to induce phenotypes that mimic accelerated aging. So far, the evidence indicates that fundamental aging processes are conserved

across species, suggesting that addressing these mechanisms could work across different segments of the human population. At the same time, interventions such as rapamycin and acarbose have shown sex differences, and even different senolytics work on different types of senescent cells. Thus, the universalist approach must be balanced with the individualist perspective, for example through tests that might predict which patients might benefit most from which intervention.

The Geroscience Network has ideas with respect to forming networks of clinical and translational centers that can conduct multicenter studies. How best to implement these ideas is under discussion. Planning and development, software development, toolkits, and collaboration are needed. Similarly, how best to implement parallel grant submissions is not yet clear. However, NIA staff are supportive. In addition, the NIA has been allowing applications with multiple principal investigators (PIs) since 2000 and tracking them carefully. Approximately 20% of awards now have multiple PIs. Whereas once the most successful applications were from single PIs with postdoctoral fellows, the most successful applications in 2015 had at least three PIs.

Translational grant applications often suffer in study sections because of a perception that the proposed scientific concept will not translate. Yet the vast majority of reviewers in these study sections have not conducted translational research and therefore are not qualified to make that determination. While the emphasis on translation is an important one, it is still viewed as a mandate from NIH. More of an open mind is needed. Likewise, a balance must be maintained between descriptive or mechanistic research and clinical trials of high-level, lead-stage interventions. Most major advances arise from descriptive research.

NIA Research Directions: The Continuing Value of Clinician Investigstors

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Clinician investigators continue to be valuable in aging research because of their experience in technology and technology development for assessing human biology and function in vivo, experience in and ability to collaborate with preclinical research, ongoing disease-focused research on the mechanisms underlying aging, knowledge of real-world constraints on the feasibility of translating an intervention, and their ability to assess the whole patient as opposed to a target organ. Over the next decades, as the U.S. population ages, specialty clinician investigators will contribute to an evolution of progress in several areas, such as:

The role of aging mechanisms in age-related conditions. Specialty clinician investigators will contribute to the development of mechanism-proximal markers and targets and relating them to multiple aging phenotypes. They also will contribute to knowledge about favorable and unfavorable clinical effects of interventions targeting particular aging mechanisms and pleiotropy in the effects of aging mechanisms on multiple outcomes. They will apply new technologies, such as imaging and other noninvasive measures, and they will illustrate the value of interspecialty collaboration.

Multimorbidity. Clinician investigators will contribute to knowledge about the bidirectional interactions between aging mechanisms and age-related conditions, how one age-related morbidity might influence the progression of others through effects on aging mechanisms, the interactions among specific diseases combinations and their treatments, and the trade-offs between the potential benefits and harms of interventions.

Prevention. Medicine is already seeing trends toward the development of interventions targeting earlier stages of a disease or risk factor, such as MCI. Clinician investigators can also contribute to knowledge about the implications of research participant selection; the development of screening methods; the potential for expanding the role of tests that might detect early stages of aging pathology; the sensitivity, specificity, and clinical impact of new screening or diagnostic tests in patients of differing ages and comorbidities; and the value of linking with epidemiological studies, and integrating information from clinical encounters with data from longitudinal studies to improve understanding of modifiable risk factors for aging outcomes.

Healthcare quality measures and evidentiary bases for payment. This area is already evolving at a rapid pace. Clinician-investigators will contribute to the development of appropriate performance and outcome measures for patients with multimorbidities and integrate them with disease-specific measures; and assess the relative clinical value of new diagnostic tests of interventions, compared with alternatives, and evidence-based indications for which patients should receive them.

All these areas will not only evolve, they will also influence each other in many ways.

Discussions throughout this meeting have emphasized the value of interspecialty collaboration in aging research. Despite its value, however, such research is hampered by real-world constraints, such as competing priorities for department chairs and section chiefs. Yet, stimulating interspecialty collaboration could prove powerful and lead the nation in revolutionizing care, particularly because of the pleiotropic nature of aging-related mechanism. GEMSSTAR awardees are therefore encouraged to share their ideas on how to promote such collaboration. In addition, as they embark upon their careers, GEMSSTAR awardees are reminded to embrace flexibility, to take advantage of unexpected developments, and to envision how research can evolve beyond the next short-term study.

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Conflicts of Interest

1. CC was a speaker for Emergency Medical Abstracts and Best Evidence in Emergency Medicine. CC also serves as a Board Member for Schwartz-Reisman Emergency Medicine Institute International Advisory Board Chair, Deputy Editor-in-Chief Academic Emergency Medicine and Associate Editor Journal of the American Geriatrics Society.

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Author Contributions

Authors JB, NB, CC, RD, LF, AH, JI, DK, GK, JLK, NM, JN, TR, AS, SS, JW and RY worked on concept and design of this manuscript. Authors AH, JI, DK worked on analysis and interpretation of data of the manuscript. Authors JB, NB, CC, RD, LF, AH, JI, DK, GK, JLK, FM, NM, JN, TR, AS, SS, JW and RY worked on the preparation of the manuscript.

Dedication

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REFERENCES

1. Varadhan R, Yao W, Matteini A, et al. Simple biologically informed inflammatory index of two serum cytokines predicts 10 year all-cause mortality in older adults. *The journals of gerontology. Series A, Biological sciences and medical sciences*. Feb 2014;69(2):165-173.

2. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *The journals of gerontology. Series A, Biological sciences and medical sciences*. May 2002;57(5):M326-332.

3. Cesari M, Penninx BW, Pahor M, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *The journals of gerontology. Series A, Biological sciences and medical sciences*. Mar 2004;59(3):242-248.

4. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *The American journal of medicine*. May 1999;106(5):506-512.

5. Binkley NC, Sun WH, Checovich MM, Roecker EB, Kimmel DB, Ershler WB. Effects of recombinant human interleukin-6 administration on bone in rhesus monkeys. *Lymphokine and cytokine research*. Aug 1994;13(4):221-226.

6. Walston J, Hadley EC, Ferrucci L, et al. Research agenda for frailty in older adults: toward a better understanding of physiology and etiology: summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *Journal of the American Geriatrics Society*. Jun 2006;54(6):991-1001.

7. Burks TN, Andres-Mateos E, Marx R, et al. Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. *Science translational medicine*. May 11 2011;3(82):82ra37.

8. Harris MI, Flegal KM, Cowie CC, et al. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994. *Diabetes care*. Apr 1998;21(4):518-524.

9. Halter JB, Musi N, McFarland Horne F, et al. Diabetes and cardiovascular disease in older adults: current status and future directions. *Diabetes*. Aug 2014;63(8):2578-2589.

10. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science*. May 16 2003;300(5622):1140-1142.

11. Rera M, Clark RI, Walker DW. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*. Dec 26 2012;109(52):21528-21533.

12. Vina J, Gomez-Cabrera MC, Borras C, et al. Mitochondrial biogenesis in exercise and in ageing. *Advanced drug delivery reviews*. Nov 30 2009;61(14):1369-1374.

13. Skyler JS. Diabetic complications. The importance of glucose control. *Endocrinology and metabolism clinics of North America*. Jun 1996;25(2):243-254.

14. Kuchel G. Aging and homeostatic regulation. In: Halter JB, Ouslander JG, Studenski S, et al., eds. *Hazzard's Geriatric Medicine and Gerontology*. 7th ed: McGraw-Hill Education; 2017.

15. Fink RI, Kolterman OG, Griffin J, Olefsky JM. Mechanisms of insulin resistance in aging. *The Journal of clinical investigation*. Jun 1983;71(6):1523-1535.

16. Young JB, Rowe JW, Pallotta JA, Sparrow D, Landsberg L. Enhanced plasma norepinephrine response to upright posture and oral glucose administration in elderly human subjects. *Metabolism: clinical and experimental*. Jun 1980;29(6):532-539.

17. Pascualy M, Petrie EC, Brodkin K, Peskind ER, Veith RC, Raskind MA. Effects of advanced aging on plasma catecholamine responses to the cold pressor test. *Neurobiology of aging*. Nov-Dec 1999;20(6):637-642.

18. Varadhan R, Seplaki CL, Xue QL, Bandeen-Roche K, Fried LP. Stimulus-response paradigm for characterizing the loss of resilience in homeostatic regulation associated with frailty. *Mechanisms of aging and development*. Nov 2008;129(11):666-670.

19. Hadley E, Kuchel GA, Newman AB, et al. Report: NIA workshop on measures of physiologic resiliencies in human aging. *The journals of gerontology. Series A, Biological sciences and medical sciences*. 2017;In press.

20. Carter BL, Ernst ME, Cohen JD. Hydrochlorothiazide versus chlorthalidone: evidence supporting their interchangeability. *Hypertension*. Jan 2004;43(1):4-9.

21. Shannon RP, Wei JY, Rosa RM, Epstein FH, Rowe JW. The effect of age and sodium depletion on cardiovascular response to orthostasis. *Hypertension*. May 1986;8(5):438-443.

22. Partridge TA, Sloper JC. A host contribution to the regeneration of muscle grafts. *Journal of the neurological sciences*. Sep 1977;33(3):425-435.

23. Sampaolesi M, Blot S, D'Antona G, et al. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*. Nov 30 2006;444(7119):574-579.

24. Cossu G, Previtali SC, Napolitano S, et al. Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. *EMBO molecular medicine*. Nov 05 2015;7(12):1513-1528.

25. Boutet SC, Disatnik MH, Chan LS, Iori K, Rando TA. Regulation of Pax3 by proteasomal degradation of monoubiquitinated protein in skeletal muscle progenitors. *Cell*. Jul 27 2007;130(2):349-362.

26. Brack AS, Conboy IM, Conboy MJ, Shen J, Rando TA. A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. *Cell stem cell*. Jan 10 2008;2(1):50-59.

27. Boutet SC, Biressi S, Iori K, Natu V, Rando TA. Taf1 regulates Pax3 protein by monoubiquitination in skeletal muscle progenitors. *Molecular cell*. Dec 10 2010;40(5):749-761.

28. Cheung TH, Quach NL, Charville GW, et al. Maintenance of muscle stem-cell quiescence by microRNA-489. *Nature*. Feb 23 2012;482(7386):524-528.

29. Boutet SC, Cheung TH, Quach NL, et al. Alternative polyadenylation mediates microRNA regulation of muscle stem cell function. *Cell stem cell*. Mar 2 2012;10(3):327-336.

30. Rodgers JT, King KY, Brett JO, et al. mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). *Nature*. Jun 19 2014;510(7505):393-396.

31. Quarta M, Brett JO, DiMarco R, et al. An artificial niche preserves the quiescence of muscle stem cells and enhances their therapeutic efficacy. *Nature biotechnology*. Jul 2016;34(7):752-759.

32. Conboy IM, Rando TA. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. *Developmental cell*. Sep 2002;3(3):397-409.

33. Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-mediated restoration of regenerative potential to aged muscle. *Science*. Nov 28 2003;302(5650):1575-1577.

34. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*. Feb 17 2005;433(7027):760-764.

35. Villeda SA, Luo J, Mosher KI, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature*. Aug 31 2011;477(7362):90-94.

36. Brack AS, Conboy MJ, Roy S, et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science*. Aug 10 2007;317(5839):807-810.

37. Carlson ME, Conboy MJ, Hsu M, et al. Relative roles of TGF-beta1 and Wnt in the systemic regulation and aging of satellite cell responses. *Aging cell*. Dec 2009;8(6):676-689.

38. Smith LK, He Y, Park JS, et al. beta2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. *Nature medicine*. Aug 2015;21(8):932-937.

39. Villeda SA, Plambeck KE, Middeldorp J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nature medicine*. Jun 2014;20(6):659-663.

40. Zhu Y, Tchkonia T, Pirtskhalava T, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging cell*. Aug 2015;14(4):644-658.

41. Miller RA. Extending life: scientific prospects and political obstacles. *The Milbank quarterly*. 2002;80(1):155-174.

42. St Sauver JL, Boyd CM, Grossardt BR, et al. Risk of developing multimorbidity across all ages in an historical cohort study: differences by sex and ethnicity. *BMJ open*. Feb 03 2015;5(2):e006413.

43. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a Tool to Target Aging. *Cell metabolism*. Jun 14 2016;23(6):1060-1065.

44. Shi Q, Liu S, Fonseca V, Shi L. Effect of metformin exposure on neurodegenerative diseases among elder adult veterans with diabetes mellitus. 76th Conference of the American Diabetes Association; 2016; New Orleans, LA.

45. Luchsinger JA, Perez T, Chang H, et al. Metformin in Amnestic Mild Cognitive Impairment: Results of a Pilot Randomized Placebo Controlled Clinical Trial. Journal of Alzheimer's disease: JAD. 2016;51(2):501-514.

46. Bannister CA, Holden SE, Jenkins-Jones S, et al. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. *Diabetes, obesity & metabolism*. Nov 2014;16(11):1165-1173.

47. Hudson KL, Lauer MS, Collins FS. Toward a New Era of Trust and Transparency in Clinical Trials. *Jama*. Oct 4 2016;316(13):1353-1354.

48. Califf RM, Zarin DA, Kramer JM, Sherman RE, Aberle LH, Tasneem A. Characteristics of clinical trials registered in ClinicalTrials.gov, 2007-2010. *Jama*. May 2 2012;307(17):1838-1847.

49. Tricoci P, Allen JM, Kramer JM, Califf RM, Smith SC, Jr. Scientific evidence underlying the ACC/AHA clinical practice guidelines. *Jama*. Feb 25 2009;301(8):831-841.

50. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *The journals of gerontology. Series A, Biological sciences and medical sciences*. Mar 2001;56(3):M146-156.

51. Garrison LP, Jr., Neumann PJ, Radensky P, Walcoff SD. A flexible approach to evidentiary standards for comparative effectiveness research. *Health affairs*. Oct 2010;29(10):1812-1817.

52. Tunis SR, Stryer DB, Clancy CM. Practical clinical trials: increasing the value of clinical research for decision making in clinical and health policy. *Jama*. Sep 24 2003;290(12):1624-1632.

53. Thorpe KE, Zwarenstein M, Oxman AD, et al. A pragmatic-explanatory continuum indicator summary (PRECIS): a tool to help trial designers. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*. May 12 2009;180(10):E47-57.

54. Choudhry NK, Avorn J, Glynn RJ, et al. Full coverage for preventive medications after myocardial infarction. *The New England journal of medicine.* Dec 1 2011;365(22):2088-2097.

55. Meeker D, Linder JA, Fox CR, et al. Effect of Behavioral Interventions on Inappropriate Antibiotic Prescribing Among Primary Care Practices: A Randomized Clinical Trial. *Jama.* Feb 9 2016;315(6):562-570.

56. Bleyer A, Welch HG. Effect of three decades of screening mammography on breast-cancer incidence. *The New England journal of medicine.* Nov 22 2012;367(21):1998-2005.

57. McEvoy RD, Antic NA, Heeley E, et al. CPAP for Prevention of Cardiovascular Events in Obstructive Sleep Apnea. *The New England journal of medicine.* Sep 8 2016;375(10):919-931.

58. Du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *The New England journal of medicine.* Feb 26 2015;372(9):803-813.

59. Kent DM, Hayward RA. Limitations of applying summary results of clinical trials to individual patients: the need for risk stratification. *Jama.* Sep 12 2007;298(10):1209-1212.

60. Lamas GA, Goertz C, Boineau R, et al. Effect of disodium EDTA chelation regimen on cardiovascular events in patients with previous myocardial infarction: the TACT randomized trial. *Jama.* Mar 27 2013;309(12):1241-1250.

61. Burd CE, Gill MS, Niedernhofer LJ, et al. Barriers to the Preclinical Development of Therapeutics that Target Aging Mechanisms. *The journals of gerontology. Series A, Biological sciences and medical sciences.* Aug 16 2016.

62. Weindruch RH, Kristie JA, Cheney KE, Walford RL. Influence of controlled dietary restriction on immunologic function and aging. *Federation proceedings.* May 1979;38(6):2007-2016.

63. Mercken EM, Hu J, Krzysik-Walker S, et al. SIRT1 but not its increased expression is essential for lifespan extension in caloric-restricted mice. *Aging cell.* Feb 2014;13(1):193-196.

64. Miller RA, Harrison DE, Astle CM, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging cell.* Jun 2014;13(3):468-477.

65. Harrison DE, Strong R, Allison DB, et al. Acarbose, 17-alpha-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. *Aging cell.* Apr 2014;13(2):273-282.

66. Ferrucci L, Studenski S. Clinical problems in aging. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, eds. *Harrison's Principles of Internal Medicine,* 18th edition: McGraw-Hill Professional Publishing; 2011.

67. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell.* Jun 6 2013;153(6):1194-1217.

68. Studenski S, Perera S, Patel K, et al. Gait speed and survival in older adults. *Jama.* Jan 5 2011;305(1):50-58.

69. White DK, Neogi T, Nevitt MC, et al. Trajectories of gait speed predict mortality in well-functioning older adults: the Health, Aging and Body Composition study. *The journals of gerontology. Series A, Biological sciences and medical sciences.* Apr 2013;68(4):456-464.

70. Howlett SE, Rockwood K. New horizons in frailty: ageing and the deficit-scaling problem. *Age and aging.* Jul 2013;42(4):416-423.