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Perspective

Biomarkers of aging for the identification and evaluation of longevity interventions

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SUMMARY

With the rapid expansion of aging biology research, the identification and evaluation of longevity interventions in humans have become key goals of this field. Biomarkers of aging are critically important tools in achieving these objectives over realistic time frames. However, the current lack of standards and consensus on the properties of a reliable aging biomarker hinders their further development and validation for clinical applications. Here, we advance a framework for the terminology and characterization of biomarkers of aging, including classification and potential clinical use cases. We discuss validation steps and highlight ongoing challenges as potential areas in need of future research. This framework sets the stage for the development of valid biomarkers of aging and their ultimate utilization in clinical trials and practice.

INTRODUCTION

Organisms change in various ways with the passage of time. Some of these changes reflect the execution of a genetic program of development, while others reflect the accumulated effects of experiences, exposures, and deleterious byproducts of life: collectively, these changes comprise aging (definition proposed by this work in Table 1). In the absence of a clear consensus on the biological definition of aging prior to this work,[1] its detrimental effects are broadly thought to be mediated by the negative consequences of biological, chemical, or physical processes, such as the accumulation of molecular damage.[2] Together, these events lead to the cumulative breakdown of physiological systems, loss of resilience, increased susceptibility to disease, and ultimately mortality.[3–5] Genetic, pharmacological, dietary, and lifestyle interventions extend healthy lifespan and/or attenuate age-related functional decline in animal models,[6] suggesting that the biological processes underlying aging are amenable to modulation.[7,8] The geroscience hypothesis posits that targeting the aging process itself,[9] rather than the individual diseases of aging, may prevent, delay, or reduce the severity of many age-related diseases in parallel.[8] In turn, this approach may modulate healthspan (Table 1).[8] While the impact of interventions on longevity can be readily investigated in animal models with short lifespans, ethical, biological, and economic considerations challenge the translation of these findings to humans.[10,11] Hence, alternative means to quantify the accumulation of age-related molecular damage and clinical functional decline are required to test interventions targeting aging.[3,11] Moreover, lifespan (and its extension) alone may not be the most informative parameter in evaluating anti-aging interventions in humans; for instance, an intervention may significantly extend healthspan without a large impact on lifespan. For these reasons, the development of biomarkers that reflect the diverse biological processes underlying aging and its consequences and that are ideally also sensitive to interventions targeting aging are critically needed. Hereafter, we refer to these biomarkers as biomarkers of aging.

In the context of interventions, a biomarker is defined as a biological feature that can indicate processes of interest in a given individual. Such processes may be normal, pathologic, or in response to a given treatment or exposure.[15] The urgent need for biomarkers of aging to identify longevity interventions was recognized as early as the 1960s in response to the earlier discovery that aging is modifiable[16] (Figure 1). To address this need, a series of U.S. National Institute on Aging (NIA)-sponsored workshops and initiatives from 1981 to 2000 explored biomarkers of aging largely in animal models.[17,18] While it was previously deemed too early to constitute a definitive panel of biomarkers of aging for animal models or humans,[6,18] molecular and omic biomarkers of aging developed over the last decade represent promising candidates (Figure 1).[3,5,7,8,11] However, there is currently no consensus on evaluation and validation methods for these biomarkers,[7,19] nor is there any standardization of how such biomarkers are utilized, even in preclinical settings. To establish a foundation on which we may build biomarkers of aging up to their full potential, we formed the Biomarkers of Aging Consortium (https://www.agingconsortium.org) to engage a multidisciplinary panel with diverse expertise related to different aspects of aging biomarkers. Here, building on prior advances in the field of aging and biomarker research, we propose a framework for classifying and assessing biomarkers of aging as tools to identify and evaluate longevity interventions (Figure 2). We establish consensus on key terminology (Table 1), the classification of biomarkers from a regulatory point of view (Figure 3), the explanation of certain use cases based on existing biomarkers (Table 2) and trials (Table 3), and the assessment of biomarkers, for instance, using validated geroprotectors (Figure 4). Our goal is to take steps to address a critical unmet need[3]: the evaluation of biomarkers to assess changes in biological age (see Table 1 for definition and contrast to chronological age). Defining common ground on these foundational issues will be key to systematically validating aging biomarkers and advancing them to the clinic.

Table 1. Definition of terms utilized in this review, in order of appearance in the text

Term[a]	Definition
Aging	The process of accumulation of consequences of life, such as molecular and cellular damage, that leads to functional decline, chronic diseases, and ultimately mortality
Healthspan	The period of life prior to onset of chronic disease and disabilities of aging, i.e., in good health (extended from Kaeberlein[12])
Biomarker of aging	A quantitative parameter of an organism that either alone or in a composite predicts biological age and ideally its changes in response to interventions
Biological age	Conceptually, an individual's age defined by the level of age-dependent biological changes, such as molecular and cellular damage accumulation. In practical use, this is often summarized as a number (in units of time) matching the chronological age where the average person in a reference population shares the individual's level of age-dependent biological changes
Chronological age	An individual's age defined by time elapsed since birth
Age acceleration (age deviation)	The difference between biological age and chronological age (originally defined by Horvath[13] and typically expressed in units of time); we propose adoption of the term age deviation (AgeDev) for this concept to distinguish it from an increased rate of aging and encompass changes in both directions
Geroprotector	An agent or intervention that increases healthspan or lifespan and ameliorates [tested] biomarkers of aging (extended from Partridge et al. and Moskalev et al.[7,14])

[a]Unless otherwise noted, terms are consensus working definitions proposed in the current work.

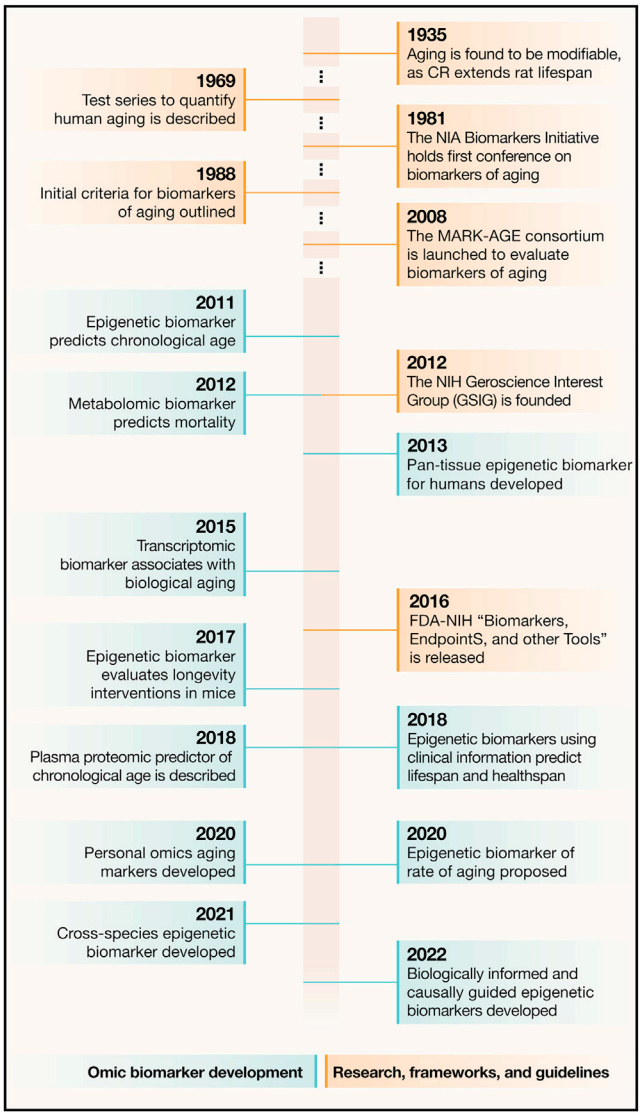

Figure 1. Timeline of events related to biomarkers of aging
Landmark events related to biomarkers of aging.

Terminology and conceptual considerations for biomarkers of aging

Aging biology as a field suffers from a lack of consensus on the biological nature of aging, and various scientists use the term "aging" to refer to different processes.[1,11,43] The current evidence suggests that aging involves deleterious changes associated with life and results in cumulative breakdown of multiple physiological systems.[11,44] However, the mechanisms underlying these changes are not uniform across time, cell types,[45] organ systems,[46] individuals,[11,47] or populations.[48] This renders it challenging to define a single and highly generalizable molecule, method, or assay that measures "aging," because this process involves multiple potentially discordant systems as well as the loss of their communication and interactions. Moreover, aging may be influenced by individual variabilities, such as the interplay of genetics, lifetime exposures, and other factors such as disease.[11,49] Given these complexities, many definitions of aging have been proposed. Crucially, multiple notions of biological aging may be valid for different aspects of this process. For instance, a definition that focuses on the age-related loss of health may be used by demographers and geriatricians but is less useful for biologists studying the basic mechanisms underlying aging. Similarly, different biomarkers may capture diverse aspects of aging, as has recently been proposed in cross-comparison studies investigating multiple molecular biomarkers of aging.[50–53] These issues highlight the need for further research and systematic evaluation to provide insights into the underlying mechanisms of aging and their relationships with various biomarkers of aging.

Despite these complexities, it is necessary to establish common working terminology for biomarker research. Relevant terms, definitions, and conceptual considerations are listed in Table 1.

CLASSIFICATIONS AND APPLICATIONS OF BIOMARKERS OF AGING

Classifications of biomarkers of aging

Multiple principal categories based on the types of associated measurements have been proposed for biomarkers of aging. These include molecular, biological, functional, clinical, and phenotypic biomarkers of aging.[54–56] To increase consistency with broader biomarker research, we propose to adapt and extend definitions from the U.S. Food and Drug Administration (FDA) that pertain to interventions, specifically the FDA-NIH Biomarkers, EndpointS, and other Tools (BEST) classification for biomarkers of aging. The FDA-BEST glossary was developed with the aim of harmonizing terms used in translational medicine to improve consistency and align expectations. This glossary broadly classifies biomarkers as molecular, physiological, histologic, or radiographic.[15] Molecular biomarkers of aging, perhaps the largest class of such biomarkers, can be based on omics (e.g., epigenomics, proteomics, or metabolomics) or specific individual molecules (e.g., circulating levels of interleukin-6 or insulin-like growth factor 1 or composites of blood markers). Physiological biomarkers of aging can be measures of functional performance (e.g., cardiorespiratory fitness, VO_2 max, gait speed, timed walking distance, grip strength, or cognitive function) or physical characteristics (e.g., body-mass index or weight-height ratio).[54] The FDA has accepted some of these surrogates, such as 6 min walk distance,[57] for approving therapies for disease indications. However, they are not currently included in the FDA's Table of Surrogate Endpoints, which are the basis of drug approval or licensure (https://www.fda.gov/drugs/development-resources/table-surrogate-endpoints-were-basis-drug-approval-or-licensure). Presumably, surrogates accepted by the FDA and other authorities for supporting disease indications could be used as primary endpoints for disease prevention or healthspan trials.

Other types of biomarkers are also being put forward for aging applications (Figure 2). For instance, digital biomarkers have recently been proposed. This type of biomarker is garnering increased attention due to advances in digital health technologies (DHTs), including both wearable and nonwearable technologies, that now allow individuals to directly collect data to

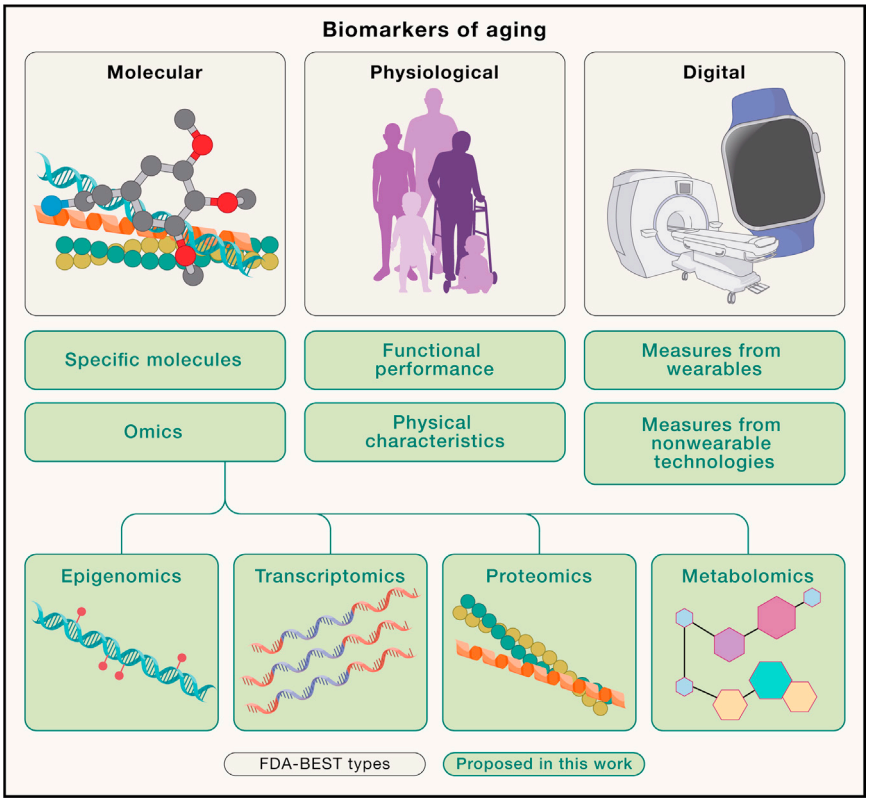

Figure 2. Common types and subtypes of biomarkers of aging based on what they measure

Gray-shaded regions are based on the broad FDA-BEST types of biomarkers framework.

explain, influence, and/or predict health- and aging-related outcomes. DHTs may thus enable large-scale measurements of biomarkers, such as longitudinal monitoring biomarkers (e.g., sleeping or moving patterns) in humans[49] and animal models.[58] Histologic and radiographic biomarkers comprise another class of tools that have not been utilized as widely in aging research for several reasons: (1) they can be more difficult to measure because they depend on either tissue biopsies or specialized equipment to visualize tissue *in situ*, both of which require expertise and time; (2) they often measure the characteristics of only specific tissues; and (3) computational methods for handling these data are less developed. These biomarkers potentially stand to become more widely used given recent advances addressing these issues.

Adapting the BEST framework, we propose a classification of common types of biomarkers of aging (Figure 2). Note that a biomarker could belong to multiple classes (e.g., molecular and physiological or functional and digital). As they are the most developed class of aging biomarkers to date, we focus mainly on molecular biomarkers of aging.

Clinical applications of biomarkers of aging

In addition to classification by type, biomarkers may also be categorized based on their clinical application. Such categories include response, predictive, investigational, mechanistic/underlying biology, surrogate, and disease outcomes.[56,59,20] Again, we propose an adaptation and extension of the FDA-BEST classification, which defines the categories of clinical susceptibility/risk, diagnostic, monitoring, prognostic, predictive, response, and safety[15] for application to biomarkers of aging. Among the listed categories of biomarkers, predictive and response biomarkers are currently the most relevant in the context of aging research, although it should be noted that no aging biomarkers of any category have been approved by U.S. regulators for clinical applications.

Predictive biomarkers

In intervention studies or clinical care, a predictive biomarker can enrich or identify individuals who may be most likely to experience beneficial or detrimental effects from a certain treatment or exposure.[15] The term is also occasionally used in epidemiological research to describe biomarkers that help to identify individuals more likely to experience a certain event (e.g., death) than others in the absence of intervention (Figure 3A). In 2019, the NIA Predictive Biomarkers Initiative (https://www.predictivebiomarkers.org) was launched to aid the development and validation of both existing and novel predictive biomarkers for age-related disease, with core goals of analytical validation of high-throughput assays and evaluation of associations of outcomes in longitudinal studies. Additionally, many recent studies have applied various biomarkers of aging to predict healthspan, lifespan, or other age-related conditions and diseases (see Table 2 for a selected list with commercial applications). For an additional list of proposed biomarkers of aging, we refer readers to the MARK-AGE project,[60] the Digital Aging Atlas (https://www.ageing-map.org/), and the TAME Biomarkers Workgroup.[59]

Prognostic biomarkers

Recent studies have also explored the potential application of biomarkers of aging as prognostic biomarkers of age-related diseases.[61,62] Prognostic biomarkers are similar to predictive biomarkers but are applied in already diseased individuals to predict disease course and/or future outcomes. For instance, some biomarkers developed broadly in the context of aging have been shown to be associated with progression of some age-related diseases, such as Alzheimer's disease[30] and cancer.[61,62] On the other hand, specific prognostic biomarkers of individual age-related diseases have been increasingly identified and tested (e.g., plasma phosphorylated-tau181 as a prognostic biomarker of Alzheimer's disease[63]). However, few studies have proposed or developed prognostic biomarkers of aging to predict progression or outcomes of the aging process as a whole.

Response biomarkers

A response biomarker indicates the biological reaction of an individual to an exposure or intervention. Response biomarkers

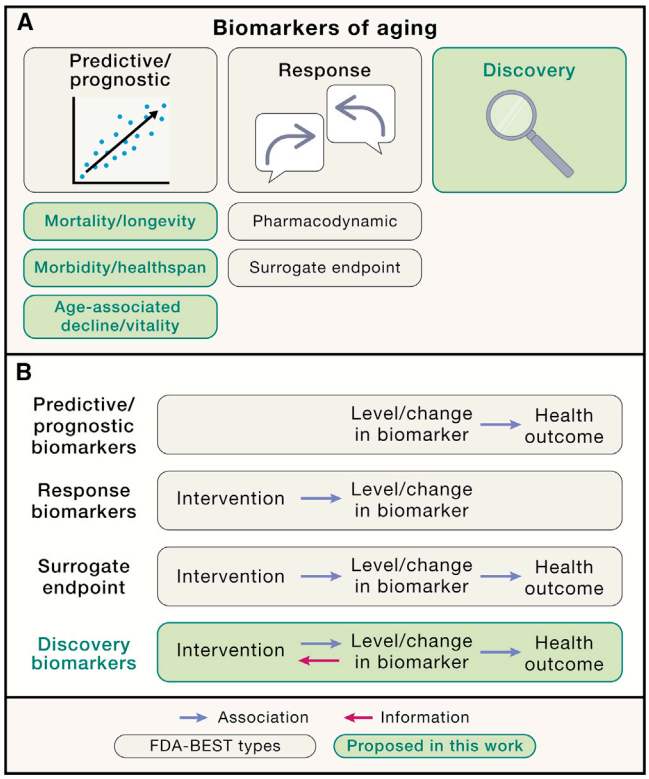

Figure 3. Categories of common and potential biomarkers of aging based on their application

(A) Classification of biomarkers of aging. Gray-shaded and green-shaded regions are based on the broad categories of biomarkers proposed by FDA-BEST and by this work, respectively.

(B) Relationships between biomarkers, interventions, and health outcomes, extended from Cummings and Kritchevsky[20] to include discovery biomarkers and the information feedback loop.

may indicate uptake or metabolism of a drug (pharmacodynamic biomarker) or a change in a biological pathway caused by the intervention.[15] Such biomarkers may be used to establish proof-of-concept, assist in dose selection, or measure the response to medical products or environmental agents.[15] While being responsive to interventions is a key criterion for aging biomarkers[59] (see also discussion of "Response criteria" below), few composite response biomarkers of aging have been identified. The Healthy Aging Index,[64] a composite score of physiologic aging, could be considered as a candidate response biomarker of aging as it not only predicts aging outcomes (e.g., mortality) but also captures response to healthy aging interventions (e.g., weight loss by caloric restriction).[59] Response biomarkers in pathways associated with an outcome may become candidate surrogate endpoint biomarkers predictive of a clinical outcome.

Surrogate endpoint biomarkers

After appropriate validation, surrogate endpoint biomarkers may be used in clinical trials as a substitute for a direct measure of how a patient or participant feels, functions, or survives[15] (Figure 3). In other words, they may serve as the primary efficacy endpoints in large, well-controlled trials intended to support regulatory approval of an intervention. For instance, blood pressure reduction is an FDA-validated surrogate endpoint for reduction in rates of stroke.[15,65] Surrogate endpoints are particularly useful when the actual desired clinical endpoint is difficult to measure or expected to manifest long after an intervention is initiated. For this reason, surrogate endpoints are highly relevant to aging, where age-related disease(s) of interest or mortality would be primary endpoints.[11,15,20] However, FDA acceptance of a surrogate endpoint is considerably challenging. Based on how well a potential surrogate endpoint is validated, U.S. regulation recognizes "validated," "likely," or "candidate" surrogate endpoints. Clinical trials are needed to show that surrogate endpoints are predictive of or correlate with clinical outcomes. Establishing that a biomarker is responsive to an intervention (response biomarker) is the first step toward identifying a surrogate endpoint biomarker. This is not the only requirement, and examples of failed surrogate endpoints exist.[20] There are not yet any formally validated or likely surrogate endpoint biomarkers of aging—in part because they have only recently been described. The challenge of validating surrogate endpoints is compounded by the lack of consensus on interventions that improve clinical outcomes relevant to healthspan or lifespan (see "Response criteria"). The correlation of candidate surrogate endpoints with clinical responses to effective interventions is generally required for full validation. Nonetheless, the investigation of biomarkers of aging as response and potential candidate surrogate endpoint biomarkers has rapidly increased over recent years.[59,66,67] In particular, epigenetic biomarkers commonly termed "clocks" have been increasingly used as candidate biomarkers of aging in clinical trials with a focus on longevity or rejuvenation. Table 3 lists recently completed or ongoing registered clinical trials that evaluate epigenetic aging signatures as response biomarkers either as predefined outcome measures or during *post hoc* analyses. Further studies will be required to confirm whether these biomarkers are associated with, or are predictive of, later clinical outcomes and thereby represent candidate surrogate endpoint biomarkers.

Discovery biomarkers

Biomarkers of aging that can be linked to biological pathways may provide practical utility for the identification of novel therapeutic targets and longevity interventions (Figure 3B). Additionally, it has been demonstrated that the use of large-scale omics data in combination with artificial intelligence models can aid in the identification of novel targets.[68] Once validated, such discovery biomarkers, possibly in combination with computational models, may reduce the prohibitive cost and time of the drug discovery process for diseases of aging.

CRITERIA FOR THE ASSESSMENT OF BIOMARKERS OF AGING

Over the past decades, several criteria for ideal biomarkers of aging have been proposed[10,17,66,69]: (1) measurement of the biomarker should be minimally invasive and reliable, i.e., it should be possible to conduct longitudinal measurements with little technical variability; (2) the biomarker should be relevant to aging; (3) the biomarker should predict functional aspects of aging, e.g., mortality, better than chronological age; and (4) the biomarker should be responsive to longevity interventions. We

Table 2. A select list of human predictive biomarkers of aging associated with various age-related conditions and their commercial applications

Biomarker of aging	Biomarker type	Age-related conditions	Commercial application[a]
DNAmAge (Horvath,[13] Hannum[21])	Epigenetic clocks, based on a set of DNA methylation measures associated with chronological age	Associated with multiple aging diseases and time-to-death, based on meta-analyses[22,23]	Licensed for estimating chronological age
GlycanAge[24]	A panel of molecular measures based on glycans attached to Immunoglobulin G (IgG) antibodies associated with chronological age	Associated with multiple diseases[25]	Commercially used to track responses to lifestyle changes
PhenoAge[26] and GrimAge[27]	Epigenetic clocks, based on a set of DNA methylation measures associated with "clinical phenotypic age measures" (a panel of age-associated molecular and physiological biomarkers, measured in blood)	Higher association with multiple aging-related diseases and time-to-death, compared to previous DNAm biomarkers, and associated with healthspan;[26,27] associated with multiple age-related clinical phenotypes (walking speed, frailty, and cognitive functions)[28]	Licensed for optimizing life insurance
DunedinPoAm and DunedinPACE[29]	Epigenetic clocks, based on a set of DNA methylation measures associated with "pace of aging measures" (a panel of age-associated molecular and physiological biomarker measurements of different organ systems)	Associated with the incidence of multiple chronic diseases, including dementia, disability, and mortality[29,30]	Licensed for tracking the rate of aging
Multi-omic biological age estimation based on KDM (Klemera-Doubal method)[31]	KDM applied to over 900 principal component transformed biomarkers (metabolites, proteins, genomics, and clinical measures)	Positively and negatively modulated by "healthy" and "unhealthy" behaviors/health conditions (e.g., type 2 diabetes), respectively[31]	Licensed for tracking biological age
Aging.AI, Deep Transcriptomic and Proteomic Clocks	AI-based blood clocks, based on hematological parameters and transcriptomic and proteomic data	Associated with all-cause mortality[32] and muscle wasting[33]	Commercially available for use in clinical trials

[a]See Table S1 for commercial application details.

expand on these concepts below. Importantly, the criteria explored here are, as a whole, neither necessary nor sufficient for the validation of biomarkers of aging. Rather, they represent a framework for the characterization and assessment of aging biomarkers to assess the extent to which a candidate biomarker may be feasible, valid, and useful for a specific context of use. As mentioned above, it may be unrealistic to identify a single biomarker that captures all aspects of biological aging and satisfies all criteria. Each biomarker of aging has advantages and limitations, which may be evaluated using this framework.

Feasibility and validity

To allow for repeated measurements in animals during studies of longevity interventions and a subsequent translation to human trials at later stages, the feasibility criteria state that the measurement should be (1) nonlethal to model animals and minimally invasive to humans, (2) repeatable (to allow for monitoring in longitudinal studies), and (3) measurable during a short time relative to the organism's lifespan.[10,17,70] The criterion of *non-age-accelerating*—i.e., the act of measuring the biomarker itself should not accelerate biological aging—has additionally been proposed for biomarkers of aging.[18] Feasibility criteria are relatively straightforward to establish, as they relate to the practical aspects of making biological/clinical measurements.

Criteria of validity comprise a more complicated set of considerations, which we explore in detail below. Briefly, the first validity criterion posits that a biomarker of aging should be age-sensitive.[17] While this may appear trivial, some considerations are warranted when applied to biomarkers of aging: for instance, a biomarker perfectly correlated with chronological age would be ideal for purposes such as forensics, but it may be less informative for assessment of longevity interventions (i.e., "paradox of biomarkers").[71] Furthermore, a recent study suggested that when the performance of a biomarker of chronological age approaches near-perfect predictive accuracy, its association with mortality attenuates.[71] Therefore, a biomarker of aging suitable

Table 3. A list of recently completed or ongoing registered clinical trials or *post hoc* analyses using epigenetic biomarkers of aging with a focus on longevity

Type[a]	Study	Intervention	Title	Design, N, age range, (m/f)	Primary outcome measure	Biomarker	Biomarker outcome measure	Result
Lifestyle	CALERIE	Caloric restriction for 2 years	Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy	RCT, 218, 21–50	Change in core body temperature and metabolic rate at 24 months compared to baseline	DunedinPACE, GrimAge, PhenoAge (blood chemistry), Horvath and Hannum clocks	*Post hoc* analysis	Significant reduction of DunedinPACE and PhenoAge (blood chemistry), no significant effects for other biomarkers of aging[34,35]
	DAMA	Plant-food-rich diet, exercise	Diet Exercise and Mammography Trial	RCT, 219, 50–69 (f)	Change in mammographic breast density	GrimAge	*Post hoc* analysis	Dietary intervention: 0.66 years ↓ (GrimAge)[36]
	MDL	Diet, exercise, stress management, phytonutrient and probiotic supplements	Methylation Diet and Lifestyle Study	RCT, 44, 50–72 (m)	Health-related quality of life	Horvath clock	Exploratory	3.2 years ↓[37]
	TirolGESUND	Intermittent fasting or smoking cessation	TirolGESUND: General Exercise, Smoking Undone, and Nutrition Diet	BCS, 156, 30–60 (f)	Epigenetic biomarkers of aging and disease risk	WID-REA, -RIA, pcgtAge, and WID-SOLA	Primary	Not yet reported
Pharma-cological	Dasatinib/ Quercetin	Dasatinib and quercetin	Safety and Effectiveness of Quercetin & Dasatinib on Epigenetic Aging	BCS, 25*, >40	Epigenetic clock	DNAm (exact biomarker not defined)	Primary	Not yet reported
	RAPA	Rapamycin	Topical-RAPA Use in Inflammation Reversal and Re-setting the Epigenetic Clock	RCT, 50*, 65–95	Epigenetic clock	Horvath clock	Primary	Not yet reported
	SGLT2i	Dapagliflozin	SGLT2 Inhibition in Older Obese Adults With Pre-diabetes	RCT, 20*, >60	Advanced glycation end products in urine	DNAm (exact biomarker not defined)	Secondary	Not yet reported
	TRIIM-X	Growth hormone for 1 year	Thymus Regeneration, Immunorestoration, and Insulin Mitigation Extension	RCT, 85*, 40–80	Epigenetic clock, thymus regeneration	GrimAge	Primary	Not yet reported

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Table 3. *Continued*

Type[a]	Study	Intervention	Title	Design, N, age range, (m/f)	Primary outcome measure	Biomarker	Biomarker outcome measure	Result
Plasmapheresis	PLASMA	Young plasma	The Plasma for Alzheimer SymptoM Amelioration (PLASMA) Study	BCS, 18, 60–95	Adverse effects as a measure of safety and tolerability	GrimAge, Horvath, Hannum, and Skin and Blood[38] clocks, PhenoAge, DNAmTL[39]	*Post hoc* analysis	0.86 years ↓ (GrimAge), no change in other clocks[40]
	Plasma-pheresis	Young plasma	Effects of Plasmapheresis on Aging Biomarkers	O, 41*, 40–60	Epigenetic clock	DNAm (exact biomarker not defined)	Primary	Not yet reported
	RESET-YOUTH	Young plasma	Reversing Epigenetic and Other Markers of Senescence by Transfusing Young Plasma To Older Human Subjects	BCS, 2120*, >40 (m)	Epigenetic clock	DNAm (exact biomarker not defined)	Primary	Not yet reported
Supplement	AC11	AC-11 supplement for 2 months	AC-11 Supplement and Biological Aging	BCS, 32*, >55	Epigenetic clock, telomere length	DNAm (exact biomarker not defined)	Primary	Not yet reported
	D-SUNNY	Vitamin D for 4 months	Vitamin D Supplementation in Overweight/Obese African American Adults and Youth	RCT, 74, 13–45	Cardiovascular phenotypes, dose-response	Horvath and Hannum age deviation	*Post hoc* analysis	1.85 years ↓ (Horvath age deviation) compared to placebo[41]
	NMN	Nicotinamide mononucleotide	To Evaluate the Efficacy and Safety of NMN as an Anti-ageing Supplement in Middle Aged and Older Adults	RCT, 90, 40–65	Cellular NAD^+ levels, walking test, health questionnaire	Aging.AI 3.0 calculator (https://www.aging.ai)	Exploratory	Maintenance of blood biological age compared to placebo[42]
	Rejuvant	Alpha-ketoglutarate	Rejuvant™ Safety and Biomarker Study	RCT, 100, 45–75	c-reactive protein levels	DNAm (exact biomarker not defined)	Exploratory	Not yet reported

[a]Table is ordered by intervention type (lifestyle, pharmacological, plasmapheresis, and supplement) and alphabetically. Most clinical studies to date have used epigenetic clocks such as the Horvath Clock. N, number of participants; m, male participants only; f, female participants only; RCT, randomized controlled trial; BCS, baseline-controlled study; O, observational; *, estimated.

for the evaluation of longevity interventions is expected to show a strong—but not perfect—correlation with chronological age. The implicit assumption underlying this criterion is that a biomarker of aging measures biological rather than chronological age. This assumption was the foundation of the first formally proposed definition of a biomarker of aging as a single or composite parameter capable of predicting aging-associated outcomes better than chronological age alone.[10]

Since the time of this original definition, advances in big data and machine learning have facilitated the identification of features, or composites thereof, that predict biological age. However, these data-driven approaches create new challenges in evaluating validity: in the absence of underlying biological or interpretable models, algorithms are likely to detect a mix of features that can cause aging or be caused by the aging process. Among those features that are not directly causal, some may still be relevant to biological age, but some may simply be uninformative correlates of chronological age. Depending on factors underlying study design and model development, models may also include features that are specific to a given study population but not generalizable and/or capture technical noise or batch effects driven by measurement errors. The interpretation of any composite biomarker of aging depends heavily on understanding the contribution of individual features, and biomarkers of aging that help to mechanistically understand the aging process should ideally retain only features that cause or are directly caused by aging.[72] Despite such challenges, many existing biomarkers of aging perform very well in predicting chronological age, future mortality, and possible response to interventions, but we must remain cautious about extending their interpretation as direct measures of biological aging.

Age-sensitivity criteria

Validity criteria may be further expanded into two related criteria: a biomarker of aging should (1) correlate with multiple age-sensitive features after adjusting for chronological age and (2) be a good predictor of all-cause mortality.[6,18] Adaptation of these criteria for functional states during aging, i.e., functional aging, has been formalized as predicting physiological, cognitive, and physical function in an age-coherent way, doing so better than chronological age, and predicting the years of remaining functionality better than chronological age.[18] While these criteria are not quantitative, they provide additional features against which a candidate biomarker may be assessed. At the heart of these extended criteria is the observation that aging is a multisystem process, and therefore a biomarker of aging should be an indicator of biological processes or responses relating to multiple physiological systems or should encompass a combination of various biomarkers of different physiological systems. Unsurprisingly, composite multisystem biomarkers are more robust and show a stronger correlation with other features of aging than single biomarkers of aging.[51,73,74] This is likely for two reasons. First, integrative biomarkers condense signal while minimizing measurement error and noise. Second, integrative biomarkers incorporate heterogeneous aspects of the aging process and thus tend to arrive closer to some "consensus" signal.[75] However, as mentioned above, the substance of the algorithms, including weights assigned to different biomarkers/processes/systems, is not necessarily biologically informed, and the results should accordingly be interpreted with caution.

Integrative biomarkers of systemic biological aging fall into two broad categories: (1) measures of the progress of aging, i.e., the extent of biological damage accumulation/deterioration and corresponding loss of integrity/resilience capacity of tissues and organ systems, and (2) measures of the pace of aging, i.e., the rate at which this progress accumulates. Biomarkers of the progress of aging typically estimate biological age. Variation in the progress of aging between individuals of the same chronological age is quantified as the difference between an individual's chronological age and their biomarker-estimated biological age. This is sometimes referred to as age acceleration,[13] but we propose the more informative and straightforward term "age deviation" (AgeDev; Table 1). The association of AgeDev and aging-associated outcomes such as morbidity, disability, and mortality is the established criterion for evaluating proposed metrics of biological aging.[76–79] Alternatively, biomarkers of aging could be designed to directly correspond to AgeDev by controlling for chronological age in the design process (e.g., GrimAge and DunedinPACE[29]). Notably, the criterion of age sensitivity primarily applies to biomarkers of biological age, where the biomarker should, on average, exhibit higher scores with increasing chronological age. By contrast, biomarkers of the rate of aging do not need to correlate with chronological age, although they may be considered "age-sensitive by design."

Mechanistic criteria

Mechanistic criteria relate to the underlying biology of aging. An improved understanding of the cellular and molecular "hallmarks" or "pillars" of aging, as well as an improved understanding of the nature of aging, suggests that underlying factors contribute to age-associated physiological decline and together determine aging phenotypes.[56,80] Accordingly, a valid biomarker of aging should reflect these underlying cellular and molecular processes.[81] A new generation of biologically informed (e.g., preliminary evidence from PRC2 clock[82] and deconstructed clocks[83]) or causally guided epigenetic clocks (e.g., preliminary evidence from DamAge clock[84]), as well as biomarkers of aging based on plasma proteomics (e.g., PROage[85] and the proteomic clock[86]), are examples of efforts toward more mechanistic biomarkers of aging. In addition, some longevity interventions have been found to modify omic biomarkers of aging, suggesting that these biomarkers could reflect the underlying mechanisms.[82,87]

Generalizability criteria

Generalizability broadly refers to the ability of biomarkers to function across different applications. For instance, a biomarker of aging may be specific to an individual cell type (e.g., naive $CD4^+$ T cells or oocytes), organ (e.g., liver or brain; for a recent review, see[88]), organ system (immune or nervous system), species (e.g., mouse or human), or human population or may be more broadly applicable. In line with the cellular view of aging promoted by the geroscience hypothesis and the hallmarks of aging, biomarkers that measure underlying common molecular processes of cellular aging might capture aging-associated molecular and cellular damage common to multiple cell/tissue types or species. Generalizability of biomarkers between different

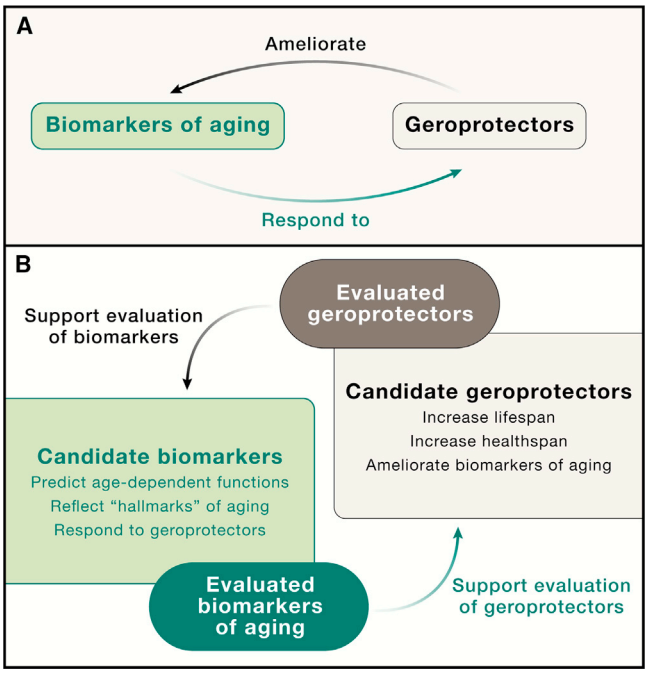

Figure 4. Relationship between biomarkers of aging and geroprotectors

(A) Biomarkers of aging and geroprotectors may appear to have a circular relationship.

(B) However, development of each is useful to the other: evaluated geroprotectors can be used to develop and benchmark biomarkers of aging, while evaluated biomarkers of aging may be used to predict or test the response to candidate geroprotectors.

tissues and between *in vivo* and *in vitro* settings may therefore be helpful to support mechanistic studies.

As many features of biological aging are conserved across multiple species,[89] aging biomarkers have also been developed for model organisms. In fact, model organisms provide an opportunity to better understand various aspects of biomarkers, including their development, validation, and application. Several epigenetic biomarkers have been developed for mice, including blood and multi-tissue epigenetic clocks.[90–92] These biomarkers were validated by analyzing dietary (e.g., calorie restriction), genetic (e.g., growth hormone receptor knockout), and pharmacological (e.g., rapamycin) interventions.[90–92] Recognizing the value of cross-species biomarkers, the American Federation of Aging Research has proposed an additional criterion of applicability in both humans and model organisms for a "true" biomarker of aging.[54,93] Examples of dual-species[94] and pan-mammalian[95] epigenetic biomarkers of age have been reported. The cross-species translatability criterion favors molecular, cellular, or subcellular level biomarkers[11] and disfavors organism-specific biomarkers such as the PhotoAgeClock, which is based on changes in human eye corners,[96] or the FRIGHT clock, which uses measures of frailty in mice.[97] However, the requirement for cross-species generalizability may not always be applicable, as there are certain aspects of biological aging that may differ between models/species. For example, telomere shortening is detected in human somatic tissues with aging but not in most strains of laboratory mice[60,98]; similarly, higher fasting blood glucose is associated with increased mortality in primates but not in mice.[99] Discarding potential biomarker candidates due to a lack of translatability may therefore result in exclusion of interesting human biomarkers.[60] However, cross-species translatability offers the advantage of applying the same biomarkers in preclinical and clinical studies.

Moving to clinical considerations, a key aspect of human aging biomarker generalizability is the ability of biomarkers to function across different populations of humans. At the very least, it should be understood what limits the wider utility of a given aging biomarker. It is thus critical to validate proposed biomarkers across countries, sexes, ethnicities, and other demographic factors. Studies in non-industrialized populations[100,101] are also crucial to establishing biomarkers as being generalizable in humans.

Response criteria

Generally, response criteria posit that a biomarker of aging should be responsive to both accelerated and decelerated aging.[59] Biomarkers of aging are expected to indicate higher age in models of accelerated aging.[102] Likewise, known factors with systemic negative effects on longevity such as chronic stress[103] are generally expected to increase scores of biomarkers of aging. Societal effects such as poverty[104] may similarly affect biomarkers of aging, though such effects may be nuanced or context-specific, which may prevent generalization or complicate interpretation. As models of accelerated aging are further developed and understood,[105] biomarkers of aging can be evaluated using these conditions. The association of conditions negatively impacting longevity with biomarkers of aging could help to further characterize these biomarkers,[106] for instance, by linking them to known underlying pathomechanisms.

At the opposite end of the spectrum, geroprotectors are agents or interventions that potentially slow, inhibit, or reverse biological aging.[7,14] For a subset of candidate geroprotectors, sufficient scientific evidence supporting their efficacy has accumulated to warrant the initiation of human clinical longevity intervention trials, such as caloric restriction (CALERIE[34]), exercise (DAMA,[36] DO-HEALTH,[107] and Generation-100[108] trials), mTOR inhibitors,[109–111] and plasmapheresis.[40] As geroprotective properties and mechanisms of certain agents and interventions are becoming more evident, their effects on biomarkers of aging are being evaluated. Geroprotectors with large effects on multiple physiological systems are expected to reduce scores of biomarkers of aging that predict biological age.

As one of the proposed criteria for a candidate geroprotector is reduction in biological age assessed with biomarkers,[7,14,69] the relationship between biomarkers of aging and geroprotectors may seem circular (Figure 4A). There exist many candidate biomarkers of aging and many proposed geroprotectors, and the accumulated evidence supporting either could be leveraged to validate the other (Figure 4B). Candidate geroprotectors may be evaluated by examining their effect on healthspan, lifespan, or evaluated biomarkers. In turn, candidate biomarkers of aging could be assessed based on their response to evaluated geroprotectors (Figure 4B). Indeed, this is currently the common

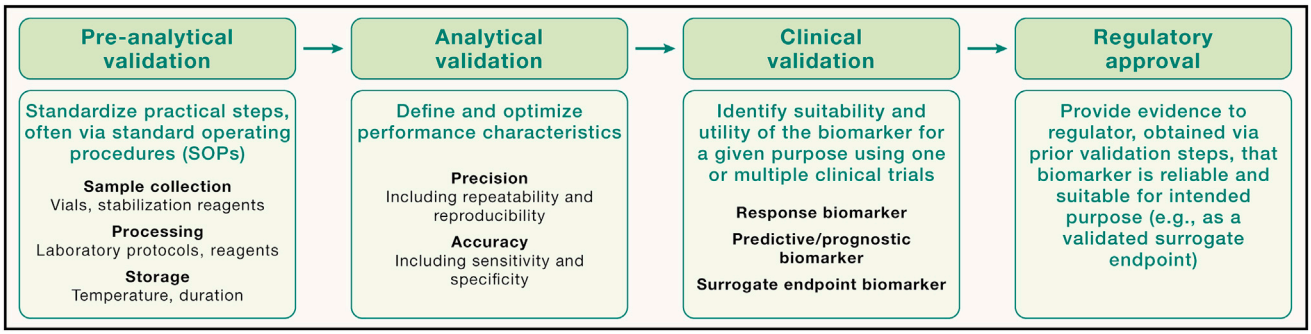

Figure 5. Analytical and clinical validation
Expanded from Dobbin et al.[114] by including details on individual validation steps.

practice for analogous biomarkers developed for use in model organisms where numerous lifespan-extending interventions have been unequivocally identified.[92]

Cost considerations

To implement biomarkers of aging in large-scale settings, including investigations of large biobanks, large clinical trials, population studies, or genome-wide screens, the cost of measurement must be relatively low. Most established biomarkers of aging currently remain cost-prohibitive for such purposes. Recent advances in cost reduction for measuring biomarkers of aging involve the development of probabilistic statistical frameworks and low-pass sequencing approaches applied to single cells and bulk samples (e.g., scAge[112]) and targeted high-throughput analyses of subsets of established biomarker loci (e.g., DNAm profiling of epigenetic clock CpG sites only, rather than unbiased or genome-wide profiling, as in preliminary evidence from TIME-seq[113]). The development of ultrahigh-throughput sequencing technologies is also expected to lead to reduced sequencing costs in the future, thus enabling the application of these and other methods in a cost-effective manner.

VALIDATION OF BIOMARKERS OF AGING

Biomarkers must undergo both analytical and clinical validation to ensure they are adequate for their intended use[114] (Figure 5). Validation is an intensive process with many factors that must be carefully considered. Here, we outline some of the key considerations for such validation efforts.

Analytical validation

It is imperative that a biomarker meets technical specifications and standards. Analytical validation seeks to determine that the biomarker and potential resulting test exhibit adequate precision.[15] Quantification of precision can be performed under repeatability (same laboratory, operator, and equipment) and reproducibility conditions (different laboratory, operator, and equipment) and can assess the agreement of independent test results.[115] These analytical aspects are essential to ensure that the error is minimal and within an acceptable range. Ultimately, this should enable test performance with high signal (biological variation) but low noise (technical variation), which is particularly important for longitudinal studies evaluating changes in biomarkers of aging in response to longevity interventions. Here, technical variability affects both the treatment and control groups, as well as both baseline and follow-up measurements. Thus, if technical variability is high in such studies, detection of biological variation may be limited.[29,116]

Additionally, analytical validation seeks to determine the accuracy of the measurement, i.e., how close the measured value is to the true value.[15,115,117] For instance, sensitivity and specificity are two common measures of accuracy,[15] but applying them to biomarkers of aging poses several challenges: there is as of yet no consensus as to how thresholds for positive or negative results could be set or how one would determine whether the true biological age was "correctly" determined. The existing analytical validation framework based on sensitivity and specificity most frequently supposes that the target information is dichotomous (e.g., old vs. young), which is not appropriate in the context of a continuous process such as aging. Further work to form a clear consensus on how to establish analytical validation of accuracy for continuous biomarkers of aging is needed.

Ultimately, for clinical implementation of a molecular biomarker of aging as a test, three essential practical components need to be considered during analytical validation[118]: (1) sampling and source materials, including biological sample collection, storage, and processing conditions (pre-analytical); (2) assays for obtaining the measurement, for instance, methylation microarrays or next generation sequencing; and (3) methods and criteria for interpreting the measurements, such as algorithms including simple thresholding, linear models, or more complex models based on deep learning. These factors must be considered as a whole and should be standardized. In the context of omic biomarkers of aging, it has been shown that individual components (e.g., DNA methylation levels for epigenetic clocks) may carry relatively high technical noise,[119] but this could potentially be overcome via computational approaches to bolster reliability[116] or judicious choice of reliable probes.[29]

Clinical validation

Clinical validity and utility are established through application in human clinical trials. Clinical validation seeks to establish whether a biomarker can indeed identify the outcome of interest and determine how useful it is for clinical decision making.[15]

Validation of a biomarker as a surrogate endpoint requires clinical evidence that a change in the surrogate endpoint predicts a specific clinical benefit.[15] For instance, a reduction in epigenetic age is only meaningful if it is indeed associated with a positive clinical change, such as a reduction in the risk of age-related diseases, frailty, mortality, or similar changes.

The process of clinical validation depends on the intended purpose of the proposed biomarker.[15] For example, predictive or prognostic biomarkers could help to estimate the magnitude of the potential benefit of a geroprotective treatment or an individual's risk of developing a specific age-related clinical outcome or death. A response biomarker of aging provides proof-of-concept that a longevity intervention has an expected biological effect. Select candidate biomarkers, such as methylation-based biomarkers, have performed well as predictive and prognostic biomarkers (Table 2) and demonstrated responsiveness to geroprotective interventions (Table 3). However, further validation is needed to establish robust associations of these biomarkers with clinical endpoints to enable their consideration as surrogate endpoint biomarkers for longevity interventions. A key step along this road is validation of biomarkers across multiple cohorts of humans. Several efforts have begun to address this need, but challenges in accessing human data while maintaining participant privacy remain and should be addressed. Nevertheless, it is increasingly clear that validated biomarkers of aging have the potential to become effective tools for clinical trials and practice.

ONGOING CHALLENGES AND DIRECTIONS FOR FUTURE RESEARCH

Biomarkers of aging have revolutionized preclinical aging biology research and stand to similarly impact clinical trials. To realize this potential, several key challenges require ongoing collective attention. These range from fundamental conceptual challenges rooted in the basic biology of aging to technical and clinical considerations, such as harmonizing terminology, classification, potential use cases, and validation steps.

At the most fundamental level, the biological nature of aging remains incompletely understood, and there is debate on which molecular, cellular, physiological, or clinical changes are causal to aging rather than simply associated with it. Disease, cumulative impacts of industrialized lifestyles, or other features—perhaps unrelated to aging entirely—underlying the sampled population may confound biomarker measurements. Sex and gender differences and their potential underlying factors must also be addressed: for instance, females tend to live longer but may experience worse health at the end of life.[120] Similarly, ethnicity as a determinant of aging should also be considered, both for people of different ethnicities within the same society and for people of the same ethnic background across different societies.[121] Finally, a host of environmental conditions and interactions between any of the aforementioned factors also likely contribute to aging. As we continue to develop our understanding of what aging is and what it is not, measurement of parameters such as vitality or resilience, which are being increasingly considered in aging studies,[122] may represent a pragmatic, complementary health-focused rather than disease-focused strategy for biomarker development. Regardless, these considerations speak to the significant need for further research aimed at understanding the biological nature of aging at a very fundamental level.

Moving to the development of aging biomarkers, a key consideration for any biomarker needs to be addressed: what assumptions are made to identify its feature(s)? Many biomarkers of aging are developed to estimate biological age by training a statistical model to predict the chronological age of the sample donor; the difference between biomarker-predicted biological age and chronological age is then referred to as the age deviation (AgeDev). However, reliance on chronological age in biomarker development may be a less than ideal strategy, as discussed above. Inclusion of relevant age-related functional parameters and outcomes to train prediction models, as implemented in recent epigenetic biomarkers (e.g., PhenoAge, GrimAge, or DunedinPACE), could improve biomarkers of aging.[11]

An important conceptual distinction needs to be made between biomarkers of biological age and biomarkers of the rate of aging. The former aim to capture the extent of aging that has occurred in an individual to date, while the latter estimate how fast aging processes are occurring at a given point in time.[123] Biomarkers of the rate of aging relate to markers of biological age as speedometers (which measure speed in distance per time) relate to odometers (which measure total distance traveled)[124,125]; thus, they may be complementary. For instance, a high AgeDev could indicate that the individual is currently aging faster and therefore has an increased biological age, or it could reflect downstream ramifications of faster aging earlier in life, even though the sampled individual is currently aging at a normal or even slower pace. Speedometer biomarkers could directly inform on the aging rate at a given point in time and may also be more sensitive to intervention-induced changes in aging processes, particularly over short follow-up intervals.[126] However, speedometer biomarkers on their own do not provide the whole picture, as they do not give an indication of the baseline biological age of the subject. These biomarkers may also be oversensitive to short-term perturbations. Hence, simultaneously measuring both AgeDev ("odometer") and the pace of aging ("speedometer") could improve prediction of future outcomes, and future research may consider their dual evaluation.

Moving to more practical considerations, the biological sample used for measuring aging is critical. For instance, biomarkers measured in blood that depend on material from cellular sources (e.g., blood cell DNA methylation or gene expression profiles) could primarily reflect changes in the hematopoietic system but not in other organ systems with lower turnover rates and different cell types. Studies have suggested that cell-type-specific aging may occur,[45,46,127,128] and hence choosing the appropriate sample type and biomarker is needed. In this regard, cell-type- and tissue-specific biomarkers may prove valuable. Moreover, the temporal dynamics, including "normal"[129] and stress-induced fluctuations,[130] of many biomarkers of aging remain to be studied, as we are only beginning to understand how to separate signal from noise in these biomarkers. For potential clinical biomarkers of aging, these challenges may be addressed by standardizing measurements of the biomarkers and clinical outcomes, banking samples using protocols focused on preserving

quality for future measurement, and reducing barriers to data access while preserving patient privacy. Furthermore, investigators should aim to apply multiple biomarkers within the same cohort. In addition to improving our understanding of fundamental aspects of aging, this would also enable further validation of biomarkers of aging and ultimately advance their clinical use.

Validation of biomarkers of aging for use as surrogate outcomes in clinical trials is highly desirable to reduce sample numbers and trial duration. An essential aspect requiring further consideration within the community will be establishing consensus on acceptable clinical outcomes for aging trials. Although a detailed discussion is beyond the scope of this perspective (for a recent review, see Cummings and Kritchevsky[20]), endpoints must be linked, in part, to the fundamental biology of aging, must be objectively quantifiable, and should evaluate the effect of an agent or intervention on how the patient or participant "functions, feels, or survives" in a manner inherently meaningful to participants (or patients), clinicians, and regulatory officials.[20] In the context of aging research, both broad (e.g., disability-free survival or incident frailty) and specific (e.g., age-related diseases or conditions) potential endpoints have been proposed to meet this definition. Total mortality is generally not considered a candidate clinical endpoint for modern trials given issues of feasibility (e.g., sample size, duration), ethics, and the conceptual challenge of disentangling all-cause vs. cause-specific death as a clinical indicator of aging as a process.[20] The latter point is important, as many biomarkers of aging are developed to predict total mortality, even though it is unlikely to serve as a primary outcome for geroprotector trials. Notably, most of the above clinical outcomes primarily occur in later life and are not ideally suited to prevention trials in young populations with low incidence of any of these outcomes. However, validated biomarkers of aging may one day enable the prediction of responsiveness to geroprotective interventions applied years before the clinical manifestations of aging.

Conclusion

Recent decades have seen considerable progress in our understanding of the aging process and how we research and quantify it. While insights into biological hallmarks of aging continue to expand,[89] it is vital to characterize what specific biomarkers of aging measure and in what settings they might find suitable applications. Biomarkers of aging offer great potential for enabling human longevity intervention trials and personalized clinical decision making. Longevity interventions may prove to be useful when applied early in life,[126,131] but given the relatively long human lifespan, trials with lifespan-focused outcomes would be impractical. Biomarkers of aging could therefore serve several important roles in aging research: (1) to give an early indication of whether an intervention increases healthspan and/or lifespan; (2) to identify individuals who might benefit from a treatment; (3) to prioritize candidate interventions for longer-term assessment; and (4) as validated surrogate endpoints for regulatory and clinical purposes if short-term changes in aging biomarkers are shown to be predictive of longer-term outcomes. Although we have approached the regulatory aspects of aging biomarkers in this work from the viewpoint of United States approval agencies, the broader considerations we have outlined are applicable to efforts to advance biomarkers across the globe. To facilitate validation and ultimate clinical application of biomarkers of aging, a robust framework for their evaluation is needed. Our hope is that the consensus terminology, classification, evaluation criteria, and identified challenges and future directions for research presented in this work represent a solid first step toward achieving this goal.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2023.08.003.

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DECLARATION OF INTERESTS

M.M., V.S., M.P.S., and V.N.G. have filed patents on measuring aging. C.H. is affiliated with the Institute for Biomedical Aging Research, Universität Innsbruck, Austria and an honorary research fellow at the Department of Women's Cancer, EGA Institute for Women's Health, University College London, United Kingdom. C.H. and M.W. are shareholders of Sola Diagnostics GmbH and named as inventors on a patent on an epigenetic clock indicative of breast cancer risk. J.N.J. is affiliated with the Sticht Center for Healthy Aging and Alzheimer's Prevention, Wake Forest University School of Medicine, Winston-Salem, NC, USA and the XPRIZE Foundation, Culver City, CA, USA. J.N.J. also serves on the advisory board for the American Federation for Aging Research (AFAR)'s Finding Aging biomarkers by Searching existing Trials (FAST) Initiative and the editorial board of Journals of Gerontology Series A Biological Sciences, eLife, and Experimental Gerontology. D.W.B. is affiliated with the Child Brain Development Network, Canadian Institute for Advanced Research and SocioMed Research Nucleus, Universidad Mayor, Santiago, Chile. D.W.B. also serves on the advisory board for the American Federation for Aging Research (AFAR)'s Finding Aging biomarkers by Searching existing Trials (FAST) Initiative and the editorial board of Journals of Gerontology Series A Biological Sciences and is an inventor of DunedinPACE, a Duke University and University of Otago invention licensed to TruDiagnostic. A.A.C. is a founder, president, and majority shareholder at Oken Health. I.B. is affiliated with the SOMT University of Physiotherapy, Amersfoort, The Netherlands and is a member of the clinical advisory board of Rejuvenate Biomed. M.W. is affiliated with the Institute for Biomedical Aging Research, Universität Innsbruck, Austria. N.B. is the Scientific Director of the American Federation for Aging Research (AFAR), on the board of the executive committee of the Longevity Biotech Association, and advisor on the Board of the Academy for Health and Lifespan Research. M.K. is an employee and shareholder of Optispan Inc., a company developing tools to enable science-based personalized and preventative geromedicine. The Regents of the University of California is the sole owner of a patent application directed at GrimAge and other epigenetic clocks for which S.H. is a named inventor. S.H. is also a founder and paid consultant of the nonprofit Epigenetic Clock Development Foundation that licenses patents surrounding epigenetic clocks. A.B.M. is the Chief Medical Officer of NU. V.N.G. is a scientific advisor to Retro and Dior. M.P.S. is a cofounder and scientific advisor of Personalis, SensOmics, Qbio, January AI, Fodsel, Filtricine, Protos, RTHM, Iollo, Marble Therapeutics, Crosshair Therapeutics, and Mirvie. M.P.S. is also a scientific advisor of Jupiter, Neuvivo, Swaza, and Mitrix. V.S. is co-founder, SAB chair, and head of research of Turn Biotechnologies.

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