REVIEW

Biomarkers for aging of blood – how transferable are they between mice and humans?

Vithurithra Tharmapalan[a,b], and Wolfgang Wagner[a,b,c*]

[a]*Institute for Stem Cell Biology, RWTH Aachen University Medical School, Aachen, Germany;* [b]*Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University Medical Faculty, Aachen, Germany;* [c]*Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD), Aachen, Germany*

Aging significantly impacts the hematopoietic system, reducing its regenerative capacity and ability to restore homeostasis after stress. Mouse models have been invaluable in studying this process due to their shorter lifespan and the ability to explore genetic, treatment, and environmental influences on aging. However, not all aspects of aging are mirrored between species. This review compares three key aging biomarkers in the hematopoietic systems of mice and humans: myeloid bias, telomere attrition, and epigenetic clocks. Myeloid bias, marked by an increased fraction of myeloid cells and decreased lymphoid cells, is a significant aging marker in mice but is scarcely observed in humans after childhood. Conversely, telomere length is a robust aging biomarker in humans, whereas mice exhibit significantly different telomere dynamics, making telomere length less reliable in the murine system. Epigenetic clocks, based on DNA methylation changes at specific genomic regions, provide precise estimates of chronologic age in both mice and humans. Notably, age-associated regions in mice and humans occur at homologous genomic locations. Epigenetic clocks, depending on the epigenetic signatures used, also capture aspects of biological aging, offering powerful tools to assess genetic and environmental impacts on aging. Taken together, not all blood aging biomarkers are transferable between mice and humans. When using murine models to extrapolate human aging, it may be advantageous to focus on aging phenomena observed in both species. In conclusion, although mouse models offer significant insights, selecting appropriate biomarkers is crucial for translating findings to human aging.

HIGHLIGHTS

- The clear myeloid bias in the hematopoietic system during aging of mice is hardly observed in humans.
- Telomere length is a better biomarker for aging in humans than in mice.
- Epigenetic clocks can be used to track the aging process in mice and human.
- Age-associated DNA methylation changes are enriched in homologous genomic regions of mice and human.

Mouse models have provided invaluable insights into the aging process due to their genetic similarity to humans, sharing approximately 99% of genes and many comparable gene expression patterns [1,2]. Aging in mice mirrors many aspects of human aging, including loss of regenerative potential, hair graying, cognitive and motor impairment, and various age-associated diseases [1]. This similarity extends to the hematopoietic system, which in both species shows reduced capacity for generation and homeostasis restoration after injury or stress [3]. Mice have a median life expectancy of only approximately 2 years compared with approximately 80 years in humans, making them useful for studying the impacts of genetics, treatments, or environmental factors on aging. However, determining the physiologic aging process in mice only by survival curves alone can be challenging and may not reflect all aspects of the aging process. Therefore, reliable biomarkers, often defined as hallmarks of aging, are urgently needed. Particularly with regard to aging research, these biomarkers should not only reflect the calendric age but also capture aspects of the individual physiologic aging process [4]. Either way, it is crucial to consider that the relevance and measurement of these aging biomarkers can differ significantly between species.

In this review, we compared the most commonly used biomarkers for aging in the hematopoietic systems of mice and humans: myeloid bias, telomere attrition, and epigenetic clocks. It is also important to acknowledge that there is a wide range of additional biomarkers for aging in blood, including upregulation of candidate genes or proteins, such as senescence-associated β-galactosidase [5], inflammatory markers [6], oxidative stress markers, DNA double-strand breaks,

Address correspondence to Wolfgang Wagner, Helmholtz-Institute for Biomedical Engineering, Institute for Stem Cell Biology, RWTH Aachen University Medical School, Pauwelsstrasse 20, Aachen, Germany; E-mail: wwagner@ukaachen.de

0301-472X/

https://doi.org/10.1016/j.exphem.2024.104600

proteomic and metabolomics profiles [7,8]. However, the three above-mentioned markers are widely used to provide semiquantitative measures for aging in blood (Figure 1) [9–16].

MYELOID BIAS OF HEMATOPOIETIC DIFFERENTIATION

The composition of blood undergoes significant changes with age, serving as a surrogate marker for the aging process [17]. Aging hematopoietic stem cells (HSCs) exhibit altered homing/mobilization efficiency, diminished lymphoid potential, and enhanced myeloid differentiation potential [18]. To compensate for reduced regenerative capacity, the fraction of hematopoietic stem and progenitor cells increases with age [19,20].

A notable hallmark of aging in blood is the shift toward myeloid leukocytes, such as granulocytes and monocytes, at the expense of lymphoid cells (including B cells, T cells, and natural killer (NK) cells), a phenomenon known as myeloid bias (My-bi). This shift underscores the prioritization of innate immunity over adaptive immunity with age [21]. In mice, myeloid bias is well documented [22–25]. An increase in myeloid cells is associated with increased inflammation, whereas a decrease in lymphoid cells compromises the adaptive immune system, which plays a crucial role in defending against infections [23]. Although myeloid-biased HSCs are present in both young and aged mice, changes in their ratio contribute to myeloid-biased hematopoiesis [26,27]. The accumulation of My-bi HSCs in older mice is a consequence of their longer lifespan compared with lymphoid-biased HSCs, which allows them to persist and dominate the aged HSC pool [28]. The fractions of myeloid/lymphoid cells in peripheral blood may change from 25/75 at 2–3 months, 45/55 at 8–10 months, and 75/25 at 18–24 months [9]. Nonetheless, differences exist between mouse strains: aged DBA/2 mice show a more pronounced shift toward My-bi and a decrease in T cells as compared with aged C57BL/6 and BALB/c mouse strains [26]. Furthermore, there are sex-specific alterations in the composition of the immune system in the spleen [29], whereas this has often not been addressed for peripheral blood. Despite variation in myeloid bias between mouse strains, it became an important marker to track the state of physiologic aging in mice.

However, myeloid bias is less pronounced in humans compared with mice. Changes in leukocyte composition are prominent in early childhood, with lymphocytes peaking after birth, and subsequently declining in adulthood [26]. Unlike the myeloid bias observed in aged mice, which is primarily due to an increase in granulocyte and monocyte progenitors, aged human adults exhibit a decrease in lymphoid and monocyte progenitors accompanied by an increase in megakaryocyte and erythroid progenitor cells [20,30,31]. Interestingly, the proportion of myeloid versus lymphoid cells remains relatively stable in adulthood. We have recently analyzed peripheral blood of 358 human blood donors, and the fractions of myeloid and lymphoid leukocyte subsets overall hardly correlated with age

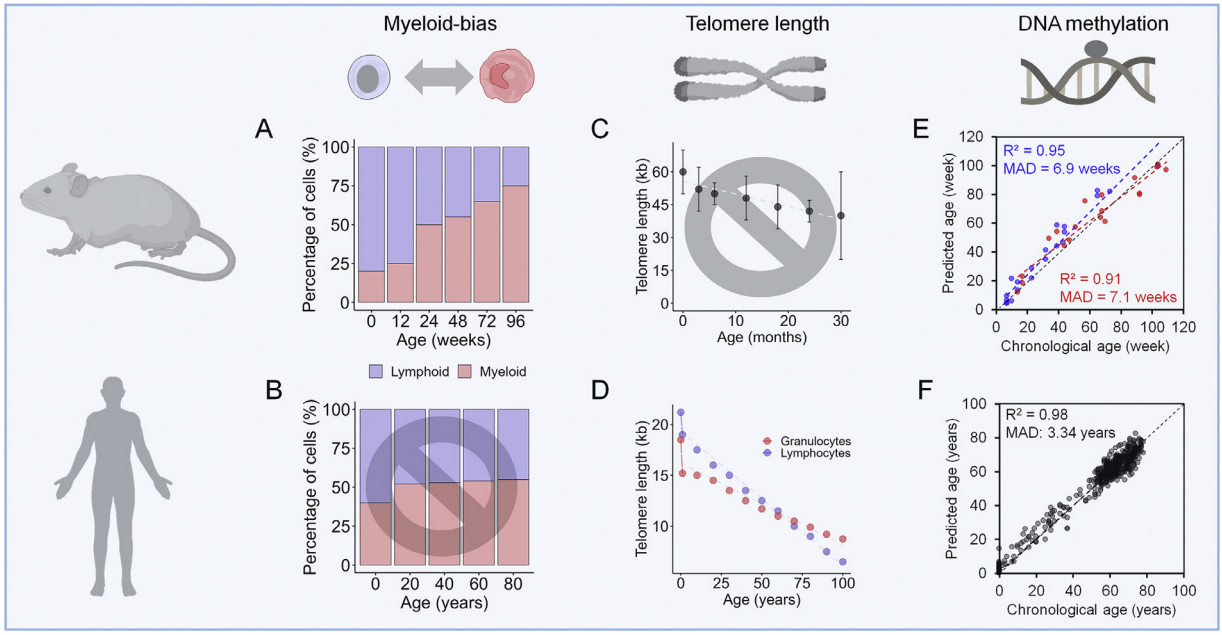

Figure 1 Schematic comparison of blood aging biomarkers in mice and humans. (**A**) Peripheral blood of mice shows a prominent shift from lymphoid cells to myeloid cells (values depicted here are just estimated from Kaschutnig et al. [9]). (**B**) In contrast, myeloid bias is hardly observed in aging humans (values are further clarified in Figure 2 [10,11]); (**C**) Telomere length is not a good indicator for biological aging in mice (average values are extrapolated from Vera et al. [12]; Whittemore et al. [13]), whereas (**D**) it declines steadily with aging in humans (average values are extrapolated from Rufer et al. [14]). (**E**) DNA methylation at three age-associated CG dinucleotides (CpGs) was used to estimate chronological age in mice in a training set (red) and an independent validation set (blue). The data were replotted from Han et al. [15]. (**F**) Exemplary presentation of epigenetic age predictions based on 101 CpGs in human blood samples (data were replotted from Weidner et al. [16]; images are derived from Biorender).

(Figure 2A–C) [10,11]. Furthermore, we used epigenetic biomarkers for the deconvolution of leukocyte subsets based on DNA methylation at specific CG dinucleotides (CpGs) [11,32]. This complementary analysis also did not reveal any pronounced differences in the ratio of myeloid versus lymphoid cells throughout adulthood (Figure 2D–F). Thus, both immunophenotypic and epigenetic characterizations demonstrate that the myeloid bias is much less pronounced in human than in mice. If humans hardly recapitulate this myeloid-biased aging phenotype of mice, it may be questionable if this phenomenon is an ideal biomarker to translate findings on the aging process from mice to humans.

Although myeloid bias is hardly observed in adult humans, there are still changes in the hematopoietic composition throughout aging. In analogy to mice, it has been demonstrated that the frequency of human HSCs in the bone marrow rather increases with age [22,33], which might compensate some loss of regenerative potential and maintain relatively high cellularity until higher ages [34]. The percentage of T cells remains relatively stable until the age of 50 years, after which it shows a significant decline. In contrast, B cell numbers rather decrease until the third decade, then increase to a peak at the sixth decade before declining [34]. NK cells decline in function and undergo phenotypic changes, however, their total numbers may hardly be affected [35]. Thus, there are changes in leukocyte counts in aging humans, but it does not recapitulate the pronounced myeloid bias in mice. Hence, myeloid bias should not be considered as an aging marker for humans.

TELOMERE LENGTH IN LEUKOCYTE SUBSETS

Telomere length is a hallmark of aging and one of the most widely used biomarkers for aging [4]. It is also frequently analyzed in hematologic malignancies, including bone marrow failure syndromes, leukemia and lymphomas [36,37]. Telomeres are repetitive sequences of "TTAGGG" nucleotides found at the ends of chromosomes, protected by a nucleoprotein complex called shelterin proteins. These structures are crucial for maintaining genomic integrity during cell replication. Telomerase, a reverse transcriptase enzyme, regulates telomere length by adding DNA repeats to chromosome ends, counteracting the natural shortening that occurs with each cell division [38,39]. Loss of telomere protection leads to gradual erosion of these caps, eventually triggering cellular senescence or apoptosis, contributing to the aging process [40]. When telomeres reach a critical length, cells undergo irreversible growth arrest, known as replicative senescence. The rate of telomere shortening is widely regarded as a contributor to organismal aging and is utilized as a biomarker for biological age prediction.

The method used to measure telomere length is crucial, as most techniques only provide average or relative measurements and may miss critical information about very short telomeres that are indicative of telomere dysfunction [41]. Flow-fluorescence in situ hybridization is considered more reliable for measuring the telomere length compared with polymerase chain reaction or luminescence-based methods, as it allows detailed analysis of telomere length distributions

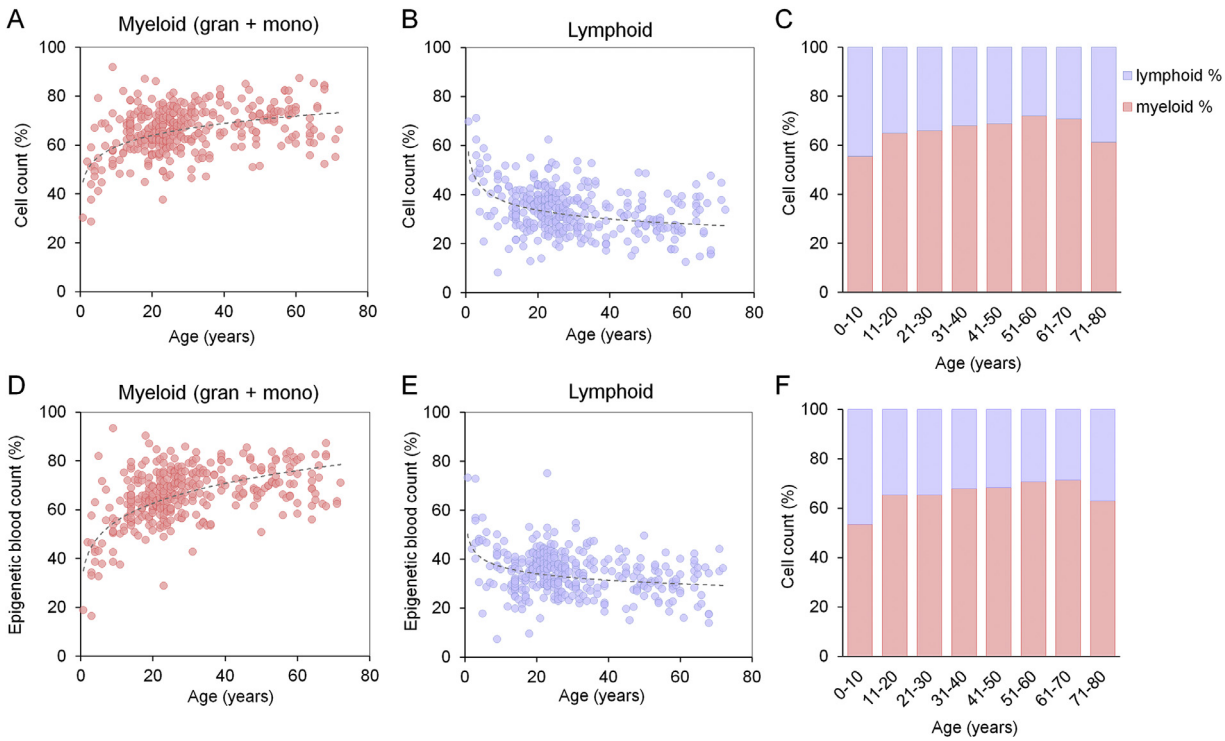

Figure 2 Analysis of myeloid bias in humans. We have replotted our data on blood counts of healthy donors (n = 348) of children [10] and adults [11]. Depicted are the automated blood counts of (**A**) the myeloid compartment (granulocytes + monocytes) and (**B**) of lymphocytes. (**C**) The fractions of myeloid/lymphoid cells hardly vary in human adults. For comparison, we have also depicted the epigenetic blood counts based on DNAm at individual CG dinucleotides (CpGs) for (**D**) granulocytes, monocytes, and (**E**) lymphocytes. (**F**) Thus, also epigenetically a myeloid bias is hardly observed in human adults. Further details on the measurements and data are provided in Hubens et al. [10,11].

within individual cells rather than just providing an average for the entire population [42,43]. Additionally, the functionality of telomeres, particularly their capping by the shelterin complex, may be more crucial than their overall length for cellular functionality [44].

In humans, telomere length in leukocytes typically decreases with age, with a rapid decline in infancy followed by a slower, more consistent decrease throughout adulthood (Figure 3A) [14,45]. However, in elderly individuals above the age of 85 years, there is no clear association between telomere length and survival or age-related diseases [46]. Different cell types exhibit varying rates of telomere length maintenance; for example, germ cells and certain adult stem cells express telomerase and maintain longer telomeres, whereas most somatic cells do not and thus have shorter telomeres [40]. HSCs from umbilical cord blood tend to have longer telomeres compared with those from adult bone marrow [47]. Lymphocytes experience a more rapid decline in telomere length with age compared with granulocytes [48], with telomere length in lymphocytes decreasing from approximately 11 kb at birth to an average of 5 kb in older individuals [43]. Shorter telomere length in leukocytes has been associated with a small increase in overall mortality risk, whereas specific diseases and organ-specific mortality risks show stronger associations [49]. Telomere length varies depending on the lymphocyte subtypes. Naïve T cells and B cells exhibit longer telomeres, whereas memory T cells and NK cells exhibit shorter telomeres [14,50,51]. These differences are attributed to the replication and proliferative needs of the cell types in response to antigenic stimulation and inflammatory cytokine levels [52]. Taken together, telomere length serves as a biomarker for aging in humans, particularly in blood cells, and this may even offer hints at the individual biological aging process [43].

In contrast, mice have longer telomeres, ranging from 30 to 150 kb in different tissues [53]. Thus, they are 2–15 times longer than in humans. Despite this, the lifespan of mice is approximately 30 times shorter than that of humans. However, their telomeres shorten at a rate of approximately 7 kb per year, which is 100 times faster than in humans [12]. Notably, there are significant differences in telomere dynamics between different laboratory mouse strains, which usually possess longer telomeres than mice in the wild [54]. Mice and humans have differences in the subtelomeric regions in the organization of repeat elements [55] and in the shelterin complex [56]. Furthermore, telomeres exhibit considerable heterogeneity among chromosomes in mice [57]. Studies on telomerase-deficient mice have shown signs of accelerated aging. However, this was not observed in the first generation upon telomerase knockout, but rather from the fifth generation onward with infertility and defective hematopoietic progenitor function [58]. Therefore, in mice telomere attrition is not such a strong marker for aging as it is in humans.

EPIGENETIC CLOCKS

DNA methylation (DNAm) is an epigenetic modification crucial in development, where methyl groups are added to cytosine's 5′ position, especially when adjacent to a guanine nucleotide (forming CpG dinucleotides). Since 2011, research has demonstrated that certain CpG sites undergo continuous DNAm changes with age and can be used to estimate donor age [59,60]. These modifications have been incorporated into predictive models known as epigenetic clocks, which are used widely to estimate chronologic age in various applications, including forensics and in understanding biological aging [61].

Epigenetic clocks, initially developed for human samples using Illumina BeadChip technology, have evolved with increasing data sets, newer technologies for DNAm profiling, and improved algorithms for integrating age-related DNAm changes. Notable examples include the Hannum clock for leukocytes [62] and the multitissue clock by Horvath [63]. Furthermore, our group described age predictors based on multiple CpGs, as well as optimized targeted assays for site-specific DNAm analysis (Figure 3B). Notably, both approaches facilitate age predictions with high precision [16,64]. Although all of these epigenetic clocks are usually based on the assumption that DNAm follows a linear or logarithmic trajectory, we have recently proposed aprobabilistic approach to determine epigenetic age based on 2D Kernel Density Estimates [65].

The first generation of epigenetic clocks were developed to predict chronologic age as close as possible, but even for these models it has been demonstrated that the deviation of predicted and chronologic age (delta age) was associated with all-cause mortality [66]. However, as mentioned above, the composition of leukocytes changes with age [67], and there are marked differences in the DNAm pattern of

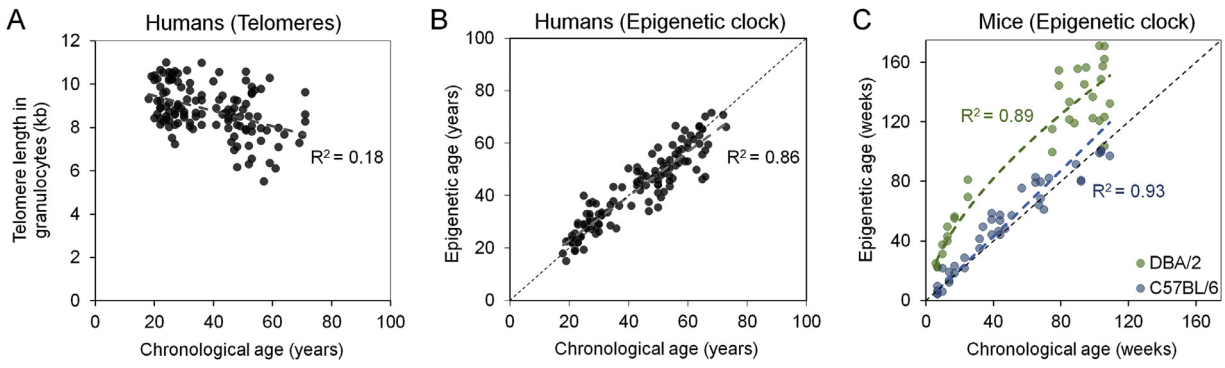

Figure 3 Age-association of telomere length and epigenetic clocks. (**A**) This figure exemplarily depicts how telomere length declines in human granulocytes with age (n = 128); measured with flow-fluorescence in situ hybridization; we have replotted data from Vieri et al. [45]. (**B**) Alternatively, we determined epigenetic age from the same samples with an epigenetic age predictor based on three CG dinucleotides (CpGs; measured with amplicon bisulfite sequencing; data replotted from Vieri et al. [45]. (**C**) Epigenetic age predictions in two different mouse strains (DBA/2 and C57BL/6) with a three CpG age predictor (data replotted from Han et al. [15]).

different leukocyte subsets [10,68]. Notably, activated cells were predicted to be older than their naïve counterparts due to different methylation patterns [69], and it has been suggested that accelerated epigenetic age (increased delta age) is primarily driven by an age-related shift in the proportion of naïve and memory immune cell composition [70]. The impact of blood counts on epigenetic age predictions is particularly observed if an epigenetic signature that has genomic regions with pronounced DNAm differences between cell types is used [69,71]. To better reflect the individual aspects of biological aging, alternative second-generation epigenetic signatures were developed, which were also trained for other clinical parameters, such as blood counts, glucose levels, or blood pressure [72]. Last but not least, third-generation clocks were generated on large cohort studies and implement additional aging parameters to better quantify individual pace of aging [73]. Thus, epigenetic clocks do capture aspects of the biological aging process.

Epigenetic clocks seem to tick rather cell intrinsically, because after allogeneic transplantation the environment of the recipient hardly impacts the epigenetic age of the transplant [74,75]. In cancer, epigenetic clocks are often accelerated, potentially because the epigenetic pattern is captured from the tumor-initiating cell [45]. Notably, in many types of cancer, accelerated epigenetic aging is also associated with a worse prognosis [76] and such biomarkers can provide insights into disease progression and the impact of different treatment interventions [77–79]. Furthermore, inflammation and a wide range of other diseases may also impact epigenetic age [80].

Because Illumina BeadChip microarrays for DNAm analysis were initially only available for humans, the first epigenetic clocks for mice were either generated on reduced representation bisulfite sequencing (RRBS) or whole-genome bisulfite sequencing (WGBS) data [81–83]. Although these approaches can theoretically address all CpGs in the genome, they are often hampered by higher sequencing costs and lower coverage, which might explain why the first epigenetic clocks for mice were described 6 years after those in humans [84]. Meanwhile, Illumina BeadChip arrays are also available for mice and have been used to derive epigenetic age predictors [85]. In fact, this technology has recently even been applied for a wide range of mammals [86]. Epigenetic clocks in mice tick faster than in humans but considering the much shorter lifespan of mice, these clocks achieve a similar precision (Figure 3C). However, it also needs to be considered that inbreed mouse strains resemble genetically almost identical individuals – epigenetic age predictors that are trained for one specific mouse strain can therefore reveal significant offsets of age predictions in other mouse strains [15]. Notably, the age-associated genomic regions in mice often correspond the homologous regions that are also age associated in humans [85]. This observation suggests a high degree of conservation in age-related DNAm sites across different species and tissues, implying the existence of a shared mammalian aging program [86].

So far, it is largely unknown how these epigenetic changes are regulated. Recent reports indicated that large parts of the predictive accuracy of epigenetic clocks can be explained by stochastic processes, which may somehow be favored at specific sites in the genome [87,88]. However, findings by our group indicated that modifications in the age-associated DNAm regions can influence epigenetic age by coherently modifying other age-associated regions across the genome, potentially regulated within a network [89]. How such a network would be governed is yet unclear. It even remains unknown if age-associated DNAm actively contributes to the aging process, or if it rather reflects other modifications, e.g., by the histone code or chromatin conformation. However, as a biomarker, epigenetic clocks provide unprecedented precision to estimate donor age and they can clearly capture relevant aspects of biological aging – in mice and humans.

CONCLUSION

Although similarities exist in the hematopoietic aging processes of mice and humans, significant differences also prevail. Mice, with their notably shorter lifespans and controlled environments, may experience hematopoietic aging differently than humans. Although numerous robust markers for aging exist in blood, as indicated above, this review focuses specifically on myeloid bias, telomere length, and epigenetic clocks. In mice, myeloid bias serves as a reliable indicator of aging, whereas its applicability to humans is limited. Conversely, telomere attrition proves to be a more accurate marker of aging in humans than in mice. Epigenetic clocks demonstrate a stronger correlation with chronologic age compared with other biomarkers across both species. Furthermore, epigenetic clocks can better reflect the impact on the individual biological aging process, particularly if specifically trained for this purpose [90]. It will be interesting in the future to derive also targeted epigenetic clocks for individual CpGs that can better capture aspects of biological rather than calendric age. In fact, such CpGs have already been identified and should be further validated in the future [65,91]. Epigenetic aging has also been demonstrated to be associated with several other hallmarks of aging, including metabolic and inflammation-associated markers [80]. However, in humans, the correlation between epigenetic age-acceleration and age-adjusted telomere attrition remains relatively modest [45,92,93]. In replicative senescence, telomere length declines, whereas not all age-associated CpGs reveal epigenetic modifications [94,95]. There have even been attempts to estimate telomere length based on epigenetic profiles, which should be further validated [96]. Conclusively, aging biomarkers can capture distinct facets of the aging process and thus should be utilized in combination. Because the murine model system is often used to gain insight into human aging, it may be advantageous to use biomarkers that are equally affected in both species.

Conflict of Interest Disclosure

W.W. and V.T. are involved in Cygenia GmbH (www.cygenia.com), which can provide services for DNA methylation analysis to other scientists.

Acknowledgments

This research was supported by the Deutsche Forschungsgemeinschaft: DFG: 363055819/GRK2415 (W.W.); WA 1706/12-2 within CRU344/417911533 (W.W.); WA1706/14-1 (W.W.); and particularly by SFB 1506/1 (W.W.); and the ForTra gGmbH für Forschungstransfer der Else Kröner-Fresenius-Stiftung (W.W.) and the Federal Ministry of Education and Research (BMBF: VIP + PluripotencyScreen).

REFERENCES

1. Dutta S, Sengupta P. Men and mice: relating their ages. Life Sci 2016;152:244–8. https://doi.org/10.1016/j.lfs.2015.10.025.

2. Rydell-Tormanen K, Johnson JR. The applicability of mouse models to the study of human disease. Methods Mol Biol 2019;1940:3–22. https://doi.org/10.1007/978-1-4939-9086-3_1.
3. de Haan G, Lazare SS. Aging of hematopoietic stem cells. Blood 2018;131:479–87. https://doi.org/10.1182/blood-2017-06-746412.
4. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194–217. S0092-8674(13)00645-4.
5. Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc 2009;4:1798–806. https://doi.org/10.1038/nprot.2009.191.
6. Ho AD, Wagner W, Mahlknecht U. Stem cells and ageing. The potential of stem cells to overcome age-related deteriorations of the body in regenerative medicine. EMBO Rep 2005;6:35–8. https://doi.org/10.1038/sj.embor.7400436.
7. Hennrich ML, Romanov N, Horn P, et al. Cell-specific proteome analyses of human bone marrow reveal molecular features of age-dependent functional decline. Nat Commun 2018;9:4004. https://doi.org/10.1038/s41467-018-06353-4.
8. Poisa-Beiro L, Thoma J, Landry J, et al. Glycogen accumulation, central carbon metabolism, and aging of hematopoietic stem and progenitor cells. Sci Rep 2020;10:11597. https://doi.org/10.1038/s41598-020-68396-2.
9. Kaschutnig P, Bogeska R, Walter D, Lier A, Huntscha S, Milsom MD. The Fanconi anemia pathway is required for efficient repair of stress-induced DNA damage in haematopoietic stem cells. Cell Cycle 2015;14:2734–42. https://doi.org/10.1080/15384101.2015.1068474.
10. Hubens W, Kluge L, Seitz A, Verjans E, Rink L, Wagner W. Epigenetic leukocyte counts from dried blood spots of pediatric patients. Clin Chem 2024;70:979–99. https://doi.org/10.1093/clinchem/hvae066.
11. Hubens WHG, Maie T, Schnitker M, et al. Targeted DNA methylation analysis facilitates leukocyte counts in dried blood samples. Clin Chem 2023;69:1283–94. https://doi.org/10.1093/clinchem/hvad143.
12. Vera E, Bernardes de Jesus B, Foronda M, Flores JM, Blasco MA. The rate of increase of short telomeres predicts longevity in mammals. Cell Rep 2012;2:732–7. https://doi.org/10.1016/j.celrep.2012.08.023.
13. Whittemore K, Vera E, Martinez-Nevado E, Sanpera C, Blasco MA. Telomere shortening rate predicts species life span. Proc Natl Acad Sci U S A 2019;116:15122–7. https://doi.org/10.1073/pnas.1902452116.
14. Rufer N, Brummendorf TH, Kolvraa S, et al. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J Exp Med 1999;190:157–67. https://doi.org/10.1084/jem.190.2.157.
15. Han Y, Eipel M, Franzen J, et al. Epigenetic age-predictor for mice based on three CpG sites. Elife 2018;7:e37462. https://doi.org/10.7554/eLife.37462.
16. Weidner CI, Lin Q, Koch CM, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol 2014;15:R24.. gb-2014-15-2-r24.
17. Geiger H, de Haan G, Florian MC. The ageing haematopoietic stem cell compartment. Nat Rev Immunol 2013;13:376–89. https://doi.org/10.1038/nri3433.
18. Dykstra B, de Haan G. Hematopoietic stem cell aging and self-renewal. Cell Tissue Res 2008;331:91–101. https://doi.org/10.1007/s00441-007-0529-9.
19. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. Nat. Med. 1996;2:1011–6. https://doi.org/10.1038/s43587-024-00619-x.
20. Rundberg Nilsson A, Soneji S, Adolfsson S, Bryder D, Pronk CJ. Human and murine hematopoietic stem cell aging is associated with functional impairments and intrinsic megakaryocytic/erythroid bias. PLoS One 2016;11:e0158369. https://doi.org/10.1371/journal.pone.0158369.
21. Rossi DJ, Jamieson CH, Weissman IL. Stems cells and the pathways to aging and cancer. Cell 2008;132:681–96. https://doi.org/10.1016/j.cell.2008.01.036.
22. Beerman I, Maloney WJ, Weissmann IL, Rossi DJ. Stem cells and the aging hematopoietic system. Curr Opin Immunol 2010;22:500–6. https://doi.org/10.1016/j.coi.2010.06.007.
23. Elias HK, Bryder D, Park CY. Molecular mechanisms underlying lineage bias in aging hematopoiesis. Semin Hematol 2017;54(1):4–11. https://doi.org/10.1053/j.seminhematol.2016.11.002.
24. Pang WW, Price EA, Sahoo D, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci U S A 2011;108:20012–7. https://doi.org/10.1073/pnas.1116110108.
25. Young K, Borikar S, Bell R, Kuffler L, Philip V, Trowbridge JJ. Progressive alterations in multipotent hematopoietic progenitors underlie lymphoid cell loss in aging. J Exp Med 2016;213:2259–67. https://doi.org/10.1084/jem.20160168.
26. Cho RH, Sieburg HB, Muller-Sieburg CE. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. Blood 2008;111:5553–61. https://doi.org/10.1182/blood-2007-11-123547.
27. Nishi K, Sakamaki T, Nagasaka A, et al. Alteration of long and short-term hematopoietic stem cell ratio causes myeloid-biased hematopoiesis. Elife 2024;13:RP96880. https://doi.org/10.7554/eLife.95880.1.
28. Muller-Sieburg CE, Sieburg HB, Bernitz JM, Cattarossi G. Stem cell heterogeneity: implications for aging and regenerative medicine. Blood 2012;119:3900–7. https://doi.org/10.1182/blood-2011-12-376749.
29. Menees KB, Earls RH, Chung J, et al. Sex- and age-dependent alterations of splenic immune cell profile and NK cell phenotypes and function in C57BL/6J mice. Immun Ageing 2021;18:3. https://doi.org/10.1186/s12979-021-00214-3.
30. Ainciburu M, Ezponda T, Berastegui N, et al. Uncovering perturbations in human hematopoiesis associated with healthy aging and myeloid malignancies at single-cell resolution. Elife 2023;12:e79363. https://doi.org/10.7554/eLife.79363.
31. Mahlknecht U, Kaiser S. Age-related changes in peripheral blood counts in humans. Exp Ther Med 2010;1:1019–25. https://doi.org/10.3892/etm.2010.150.
32. Sontag S, Bocova L, Hubens WHG, et al. Toward clinical application of leukocyte counts based on targeted DNA methylation analysis. Clin Chem 2022;68:646–56. https://doi.org/10.1093/clinchem/hvac006.
33. Taraldsrud E, Grogaard HK, Solheim S, Lunde K, Floisand Y, Arnesen H. Age and stress related phenotypical changes in bone marrow CD34+ cells. Scand J Clin Lab Invest 2009;69:79–84. https://doi.org/10.1080/00365510802419447.
34. Ogawa T, Kitagawa M, Hirokawa K. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. Mech Ageing Dev 2000;117:57–68. https://doi.org/10.1016/s0047-6374(00)00137-8.
35. Hazeldine J, Lord JM. The impact of ageing on natural killer cell function and potential consequences for health in older adults. Ageing Res Rev 2013;12:1069–78. https://doi.org/10.1016/j.arr.2013.04.003.
36. Brummendorf TH, Balabanov S. Telomere length dynamics in normal hematopoiesis and in disease states characterized by increased stem cell turnover. Leukemia 2006;20:1706–16. https://doi.org/10.1038/sj.leu.2404339.
37. Ohyashiki JH, Sashida G, Tauchi T, Ohyashiki K. Telomeres and telomerase in hematologic neoplasia. Oncogene 2002;21:680–7. https://doi.org/10.1038/sj.onc.1205075.
38. Greider CW. Telomere length regulation. Annu Rev Biochem 1996;65:337–65. https://doi.org/10.1146/annurev.bi.65.070196.002005.

39. Vieri M, Brummendorf TH, Beier F. Treatment of telomeropathies. Best Pract Res Clin Haematol 2021;34:101282. https://doi.org/10.1016/j.beha.2021.101282.
40. Trybek T, Kowalik A, Gozdz S, Kowalska A. Telomeres and telomerase in oncogenesis. Oncol Lett 2020;20:1015–27. https://doi.org/10.3892/ol.2020.11659.
41. Lai TP, Wright WE, Shay JW. Comparison of telomere length measurement methods. Philos Trans R Soc Lond B Biol Sci 2018;373:20160451. https://doi.org/10.1098/rstb.2016.0451.
42. Ferreira MSV, Kirschner M, Halfmeyer I, et al. Comparison of flow-FISH and MM-qPCR telomere length assessment techniques for the screening of telomeropathies. Ann N Y Acad Sci 2020;1466:93–103. https://doi.org/10.1111/nyas.14248.
43. Vaiserman A, Krasnienkov D. Telomere length as a marker of biological age: state-of-the-art, open issues, and future perspectives. Front Genet 2020;11:630186. https://doi.org/10.3389/fgene.2020.630186.
44. Blackburn EH. Switching and signaling at the telomere. Cell 2001;106:661–73. https://doi.org/10.1016/s0092-8674(01)00492-5.
45. Vieri M, Tharmapalan V, Kalmer M, et al. Cellular aging is accelerated in the malignant clone of myeloproliferative neoplasms. Blood Cancer J 2023;13:164. https://doi.org/10.1038/s41408-023-00936-1.
46. Martin-Ruiz CM, Gussekloo J, van Heemst D, von Zglinicki T, Westendorp RGJ. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. Aging Cell 2005;4:287–90. https://doi.org/10.1111/j.1474-9726.2005.00171.x.
47. Lansdorp PM. Telomere length and proliferation potential of hematopoietic stem cells. J Cell Sci 1995;108:1–6. https://doi.org/10.1098/rstb.2016.0451.
48. Aubert G, Baerlocher GM, Vulto I, Poon SS, Lansdorp PM. Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. PLoS Genet 2012;8:e1002696. https://doi.org/10.1371/journal.pgen.1002696.
49. Schneider CV, Schneider KM, Teumer A, et al. Association of telomere length with risk of disease and mortality. JAMA Intern Med 2022;182:291–300. https://doi.org/10.1001/jamainternmed.2021.7804.
50. Andreu-Sanchez S, Aubert G, Ripoll-Cladellas A, et al. Genetic, parental and lifestyle factors influence telomere length. Commun Biol 2022;5:565. https://doi.org/10.1038/s42003-022-03521-7.
51. Lin J, Epel E, Cheon J, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B cells: insights for epidemiology of telomere maintenance. J Immunol Methods 2010;352:71–80. https://doi.org/10.1016/j.jim.2009.09.012.
52. Svenson U, Nordfjall K, Baird D, et al. Blood cell telomere length is a dynamic feature. PLoS One 2011;6:e21485. https://doi.org/10.1371/journal.pone.0021485.
53. Kipling D, Cooke HJ. Hypervariable ultra-long telomeres in mice. Nature 1990;347:400–2. https://doi.org/10.1038/347400a0.
54. Hemann MT, Greider CW. Wild-derived inbred mouse strains have short telomeres. Nucleic Acids Res 2000;28:4474–8. https://doi.org/10.1093/nar/28.22.4474.
55. Smoom R, May CL, Ortiz V, et al. Telomouse-a mouse model with human-length telomeres generated by a single amino acid change in RTEL1. Nat Commun 2023;14:6708. https://doi.org/10.1038/s41467-023-42534-6.
56. Broccoli D, Chong L, Oelmann S, et al. Comparison of the human and mouse genes encoding the telomeric protein, TRF1: chromosomal localization, expression and conserved protein domains. Hum Mol Genet 1997;6:69–76. https://doi.org/10.1093/hmg/6.1.69.
57. Zijlmans JM, Martens UM, Poon SS, et al. Telomeres in the mouse have large inter-chromosomal variations in the number of T2AG3 repeats. Proc Natl Acad Sci U S A 1997;94:7423–8. https://doi.org/10.1073/pnas.94.14.7423.
58. Calado RT, Young NS. Telomere diseases. N Engl J Med 2009;361:2353–65. https://doi.org/10.1056/NEJMra0903373.
59. Bocklandt S, Lin W, Sehl ME. Epigenetic predictor of age. PLoS. ONE. 2011;6:e14821. https://doi.org/10.1371/journal.pone.0014821.
60. Koch CM, Wagner W. Epigenetic-aging-signature to determine age in different tissues. Aging 2011;3:1018–27. https://doi.org/10.18632/aging.100395.
61. Bell CG, Lowe R, Adams PD, et al. DNA methylation aging clocks: challenges and recommendations. Genome Biol 2019;20:249. https://doi.org/10.1186/s13059-019-1824-y.
62. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell 2013;49:359–67. https://doi.org/10.1016/j.molcel.2012.10.016.
63. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14:R115. https://doi.org/10.1186/gb-2013-14-10-r115.
64. Han Y, Franzen J, Stiehl T, et al. New targeted approaches for epigenetic age predictions. BMC Biol 2020;18:71. https://doi.org/10.1186/s12915-020-00807-2.
65. Perez-Correa JF, Stiehl T, Marioni RE. Weighted 2D-kernel density estimations provide a new probabilistic measure for epigenetic age. bioRxiv 2024. https://doi.org/10.1101/2024.06.10.598169.
66. Marioni RE, Shah S, McRae AF, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol 2015;16:25. https://doi.org/10.1186/s13059-015-0584-6.
67. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. Genome Biol 2014;15:R31. https://doi.org/10.1186/gb-2014-15-2-r31.
68. Frobel J, Bozic T, Lenz M, et al. Leukocyte counts based on DNA methylation at individual cytosines. Clin Chem 2018;64:566–75. https://doi.org/10.1373/clinchem.2017.279935.
69. Jonkman TH, Dekkers KF, Slieker RC, et al. Functional genomics analysis identifies T and NK cell activation as a driver of epigenetic clock progression. Genome Biol 2022;23:24. https://doi.org/10.1186/s13059-021-02585-8.
70. Zhang Z, Reynolds SR, Stolrow HG, Chen JQ, Christensen BC, Salas LA. Deciphering the role of immune cell composition in epigenetic age acceleration: insights from cell-type deconvolution applied to human blood epigenetic clocks. Aging Cell 2024;23:e14071. https://doi.org/10.1111/acel.14071.
71. Zhang Q, Vallerga CL, Walker RM, et al. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. Genome Med 2019;11:54. https://doi.org/10.1186/s13073-019-0667-1.
72. Bernabeu E, McCartney DL, Gadd DA, et al. Refining epigenetic prediction of chronological and biological age. Genome Med 2023;15:12. https://doi.org/10.1186/s13073-023-01161-y.
73. Belsky DW, Caspi A, Corcoran DL, et al. DunedinPACE, a DNA methylation biomarker of the pace of aging. Elife 2022;11:e73420. https://doi.org/10.7554/eLife.73420.
74. Soraas A, Matsuyama M, de Lima M, et al. Epigenetic age is a cell-intrinsic property in transplanted human hematopoietic cells. Aging Cell 2019;18:e12897. https://doi.org/10.1111/acel.12897.
75. Weidner CI, Ziegler P, Hahn M, et al. Epigenetic aging upon allogeneic transplantation: the hematopoietic niche does not affect age-associated DNA methylation. Leukemia 2015;29:985–8. https://doi.org/10.1038/leu.2014.323.
76. Lin Q, Wagner W. Epigenetic aging signatures are coherently modified in cancer. PLoS Genet 2015;11:e1005334. https://doi.org/10.1371/journal.pgen.1005334.
77. Mak JKL, McMurran CE, Kuja-Halkola R, et al. Clinical biomarker-based biological aging and risk of cancer in the UK Biobank. Br J Cancer 2023;129:94–103. https://doi.org/10.1038/s41416-023-02288-w.
78. Perna L, Zhang Y, Mons U, Holleczek B, Saum KU, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenetics 2016;8:64. https://doi.org/10.1186/s13148-016-0228-z.

79. Wang S, Prizment A, Thyagarajan B, Blaes A. Cancer treatment-induced accelerated aging in cancer survivors: biology and assessment. Cancers (Basel) 2021;13:427. https://doi.org/10.3390/cancers13030427.
80. Irvin MR, Aslibekyan S, Do A, et al. Metabolic and inflammatory biomarkers are associated with epigenetic aging acceleration estimates in the GOLDN study. Clin Epigenetics 2018;10:56. https://doi.org/10.1186/s13148-018-0481-4.
81. Petkovich DA, Podolskiy DI, Lobanov AV, Lee SG, Miller RA, Gladyshev VN. Using DNA methylation profiling to evaluate biological age and longevity interventions. Cell Metab 2017;25:954–960.e956. https://doi.org/10.1016/j.cmet.2017.03.016.
82. Stubbs TM, Bonder MJ, Stark AK, et al. Multi-tissue DNA methylation age predictor in mouse. Genome Biol 2017;18:68. https://doi.org/10.1186/s13059-017-1203-5.
83. Thompson MJ, Chwialkowska K, Rubbi L, et al. A multi-tissue full lifespan epigenetic clock for mice. Aging 2018;10:2832–54. https://doi.org/10.18632/aging.101590.
84. Wagner W. Epigenetic aging clocks in mice and men. Genome Biol 2017;18:107. https://doi.org/10.1186/s13059-017-1245-8.
85. Perez-Correa JF, Tharmapalan V, Geiger H, Wagner W. Epigenetic clocks for mice based on age-associated regions that are conserved between mouse strains and human. Front Cell Dev Biol 2022;10:902857. https://doi.org/10.3389/fcell.2022.902857.
86. Lu AT, Fei Z, Haghani A, et al. Universal DNA methylation age across mammalian tissues. Nat Aging 2023;3:1144–66. https://doi.org/10.1038/s43587-023-00462-6.
87. Meyer DH, Schumacher B. Aging clocks based on accumulating stochastic variation. Nature Aging 2024;4:871–85. https://doi.org/10.1038/s43587-024-00619-x.
88. Tong HG, Dwaraka VB, Chen QW, et al. Quantifying the stochastic component of epigenetic aging. Nature Aging 2024;4:886–901. https://doi.org/10.1038/s43587-024-00600-8.
89. Liesenfelder S, Elsafi Mabrouk MH, Illiescu J, et al. Epigenetic editing at individual age-associated CpGs affects the genome-wide epigenetic aging landscape. Biorxiv 2024. https://doi.org/10.1101/2024.06.04.597161.
90. Belsky DW, Moffitt TE, Cohen AA, et al. Eleven telomere, epigenetic clock, and biomarker-composite quantifications of biological aging: do they measure the same thing? Am J Epidemiol 2018;187:1220–30. https://doi.org/10.1093/aje/kwx346.
91. Lin Q, Weidner CI, Costa IG, et al. DNA methylation levels at individual age-associated CpG sites can be indicative for life expectancy. Aging 2016;8:394–401. https://doi.org/10.18632/aging.100908.
92. Cypris O, Eipel M, Franzen J, et al. PRDM8 reveals aberrant DNA methylation in aging syndromes and is relevant for hematopoietic and neuronal differentiation. Clin Epigenetics 2020;12:125. https://doi.org/10.1186/s13148-020-00914-5.
93. Franzen J, Nuchtern S, Tharmapalan V. Epigenetic clocks are not accelerated in COVID-19 patients. Int J Mol Sci 2021;22:9306. https://doi.org/10.3390/ijms22179306.
94. Koch CM, Reck K, Shao K, et al. Pluripotent stem cells escape from senescence-associated DNA methylation changes. Genome Res 2013;23:248–59. https://doi.org/10.1101/gr.141945.112.
95. Wagner W. The link between epigenetic clocks for aging and senescence. Front Genet 2019;10:303. https://doi.org/10.3389/fgene.2019.00303.
96. Lu AT, Seeboth A, Tsai PC, et al. DNA methylation-based estimator of telomere length. Aging 2019;11:5895–923. https://doi.org/10.18632/aging.102173.