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Review

Advancing Single-Cell Detection of Senescent Cells: Laboratory Methods and Clinical Applications

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Abstract

Cellular senescence (aging) is a physiological process that plays a role in tissue remodeling, wound healing, and embryogenesis. Prolonged senescence of cells can have detrimental effects and trigger a secretory phenotype (SASP, senescence-associated secretory phenotype), degenerative disorders, cancer, and age-related diseases. Suitable biomarkers and a range of different laboratory methods are used to investigate these complex relationships in vitro and in vivo. Since a universal biomarker for cell senescence has not yet been identified, numerous biomarkers are used to identify a senescent cell. The detection and quantification of these cells and their SASP provide the basis for targeted treatment of the patient. In parallel, single-cell analysis is also required for the quantitative assessment of the therapy result. Depending on the facilities of the laboratory performing the analysis, a wide range of analysis methods are available. In this review, we provide a general overview of accessible techniques such as immunohistochemistry using microscopy and automated flow cytometry and introduce new possibilities by modern techniques like mass spectrometry or a genetic method for the

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detection of senescent single cells. The focus here is the use of these techniques in routine laboratories. The classical laboratory analysis with the use of enzyme immunoassays, such as the measurement of products of the SASP (IL-6, IL-8), is not part of this work. This review discusses new ideas for the visualisation of clinical and laboratory patient data for gerontology. An outlook on potential and future optimization possibilities to improve or rejuvenate the senescence status of patients on a cellular and organ-specific level is discussed.

Keywords
Cellular senescence; senescent cell; SASP; aging markers; single cell methods; NGS; FACS; IHC; lab-report; human aging interventions

1. Introduction

Aging is omnipresent, and dealing with the aging process is an inevitable part of life. In addition to the aging of our Western population, healthy aging is becoming increasingly important from an economic perspective. For the medical doctor, the individual aging of the patient is often easily recognizable during the physical examination because of the overall appearance. A further classification of the patient's senescence is made with the help of proven tests and standard questions that assess the physical and mental condition. In addition, available laboratory data from the general practitioners and specialists consulted in the past can provide additional support in assessing the patient's age. Medical doctors use these data to categorize patients not only chronologically according to their date of birth but also biologically with the help of age-related reference values. In general, it is often surprising that there is not always an overlap between chronological and biological age. Further research is needed to enhance the categorization of individuals into age classes, particularly at the single-cell level.

Over the past few decades, a great deal of knowledge has been gained about senescence in cell cultures. In contrast, however, the study of senescence in living, higher organisms such as humans is still incomplete [1]. One reason is the technical limitation of methods that could potentially be used for the in vivo identification and characterization of senescent cells in tissues and organs. The samples available for medical routine laboratories are typically blood samples obtained through venipuncture or, depending on the clinical question, tissue biopsies. Data from individual cell structures, such as the SASP and the senescence status of human or murine single cells, have made a valuable contribution to potential therapies for age-related diseases using the available methods in today's precision medicine [2-4].

2. Ageing and Remodelling Processes: Senescent Single Cells and Their Cellular Products

To introduce the topic of aging, only a subjective and rudimentary selection of references is listed here. The knowledge of a limited cell division up to programmed cell death (apoptosis) in eukaryotic cells, initiated by the shortening of telomeres, made us aware of a fundamental aspect of senescence [5-9]. The state of cellular senescence resulting from stress signaling characterizes a transition to an irreversible cell cycle arrest, which is distinct from the reversible cell cycle phase G0 (rest phase) [10]. Cells in a culture can no longer divide after approximately 50 cell divisions [7]. The

cellular structures responsible for this cell division arrest are telomeres, which protect the DNA ends from wear and tear [8, 9]. As telomeres progressively shorten with each cell division, a critical length is possibly reached, which is then recognized as DNA damage, thereby initiating a DNA damage response (DDR). This response culminates in the inhibition of the cell cycle [11].

In her review Senescent Cells, Tumor Suppression and Organismal Aging: Good Citizens, Bad Neighbors [12], the ageing researcher Judith Campisi (1948-2023) further demonstrated influences and cellular reactions in the aging process. Uncontrolled growth and cancer as a danger of senescence in the organism could be kept in balance by means of tumor suppressor mechanisms. The gene that codes for p53 acts as a ‘genome guardian’ in this context, as is generally known. If there is a problem for an affected cell that would have far-reaching consequences for its continued existence, this leads to p53 activation in the cell [13]. The resulting interruption of the cell cycle leads to programmed cell death, the apoptosis.

Typical examples of such problems are DNA damage and activation of oncogenes, as they do occur under physical, chemical, and biological influences or due to a lack of growth factors. Extracellular signals or even malfunctions of signals within the cell can lead to the initiation of apoptosis. Signaling for apoptotic cell death can be divided into an extrinsic apoptosis pathway, triggered by, e.g., specific receptors, and an intrinsic, also called mitochondrial apoptosis pathway, which is triggered by environmental damage, e.g., UV light. Therefore, the inhibition of p53 activation is essential for maintaining the normal state of a cell under default conditions. However, this is why p53 activation occurs as a classic side effect of chemotherapy and radiotherapy. The senescence reaction ensures that the affected cell withdraws from the cell cycle and can, therefore, no longer form a tumor by growing incorrectly. In addition to the apoptosis form, the death of individual cells is manifold and often leads directly to the death of the corresponding cell clusters. In addition to apoptosis, cellular responses to stress also include autophagy and, above all, cellular senescence.

Cellular stress responses often occur in parallel and are dependent on the type of stress. This is a general problem in our aging process. For example, physical or chemical injuries caused by a lack of oxygen during a heart attack show classic damage to the heart muscle. Brain areas or individual brain cells experience the same problems due to chemical noxae (e.g. alcohol) or a lack of oxygen and glucose. Local inflammation can also lead to necroses triggered by membrane damage to the affected cells, e.g., caused by antibodies or a complement attack [14].

Human senescent cells, induced to enter senescence by genotoxic stress, accumulate with age and are characterized by the extensive secretion of proteins, predominantly chemokines, and cytokines, which are linked to inflammation and malignancy. The term SASP, or senescence-associated secretory phenotype, is a conceptually important starting point for understanding cellular senescence and its associated pathological processes, which was established by Judith Campisi and her research group [15, 16]. The senescence-associated secretory phenotype (SASP) develops gradually over several days and only emerges following DNA damage significant enough to trigger senescence. This accumulation subsequently leads to the production of degradative enzymes as well as inflammatory cytokines and growth factors. This unwanted production is, therefore, also part of the partial disruption of the natural tissue structure in aging tissue and can influence the microenvironment, affecting both the functional formation of the cell and its morphological and structural differentiation. Another serious problem of senescent cells is their possibility to create and optimize a microenvironment for tumor development by shaping and

remodeling their environment [12, 16-18]. However, strikingly similar SASPs also developed in normal fibroblasts, normal epithelial cells, and epithelial tumor cells after treatment of prostate cancer patients [15]. Two manipulations enhanced and accelerated the development of SASPs, oncogenic RAS expression, which causes genotoxic stress and senescence in normal cells, and the functional loss of the tumor suppressor protein p53. Both the loss of p53 and the enhancement of oncogenic RAS expression also exacerbated the promalignant paracrine activities of SASPs [15].

Laboratory testing methods can detect the remodeling processes, signaling pathways, and genetic influences of senescent cells either directly or indirectly through their products, enabling the identification of aging tissues, organs, and, ultimately, individual senescent cells. These detection methods available for aged cells in routine and research departments will be described in the following paragraphs.

3. Methods and Strategies for Single-Cell Measurement

Cellular senescence is broadly understood as a stress response triggered by various factors. This response leads to cell cycle arrest, cellular remodeling, and the development of a bioactive secretome, referred to as the senescence-associated secretory phenotype (SASP). In those areas, senescent cells accumulate within tissues. Organisms activate renewable tumor suppressor proteins in response to stress stimuli. These processes present multiple potential targets for measurements and analysis in laboratory settings.

The search for the causes of aging and the biomarkers (hallmarks) available for this, especially for the immune system - Hallmarks of Immunosenescence – [19-24] has continued to gain momentum in recent decades due to new insights into pathological processes and the availability of new innovative laboratory methods (e.g. genomics, scRNA sequencing, mass spectrometry) [22]. From an analytical point of view, it is crucial to ensure the precise labeling ("targeting") of senescent cells using suitable reagents, alongside the application of appropriate qualitative and quantitative measurement methods. This task is challenging because no single marker with high sensitivity and specificity for detecting senescent single cells is currently known. In addition, the aging research field lacks a common "gold standard" for the "hallmarks of senescence" in the laboratory area [25]. There are several markers, such as p16, p21, p53, and pRb, which are expressed by senescent cells that do not proliferate [26]. Senescent cells also possess heterochromatin foci (SAHF, Senescence Associated Heterochromatin Foci), which can be detected with special markers such as H3K9me3 and HP-1 [27, 28]. However, measurements in vivo remain technically challenging, as the historical overview of the methods used shows [1, 29].

Nowadays, the laboratory has direct and indirect detection methods for the quantitative measurement of aged cells, cell clusters, and their protein products that are undergoing remodeling, senescence, or even destruction, see Figure 1. Laboratory specialists and pathologists use available methods from the fields of clinical chemistry, hematology, immunology, microbiology, and genetics to find single senescent cells, their cell regions, and their secreted products [30]. The laboratory techniques should be suitable for routine use and available for specialized laboratories, specialist practices, or clinics without additional high technical expenditure. Numerous laboratories with a focus on hematology applications utilize flow cytometry, but new technologies, such as mass spectrometry or gene sequencing for single-cell measurements, are still more commonly found in research laboratories. The reasons for this are that the examination of senescence at the single-cell

level is cost-intensive and poses great challenges for specialist staff in terms of additional specialized training.

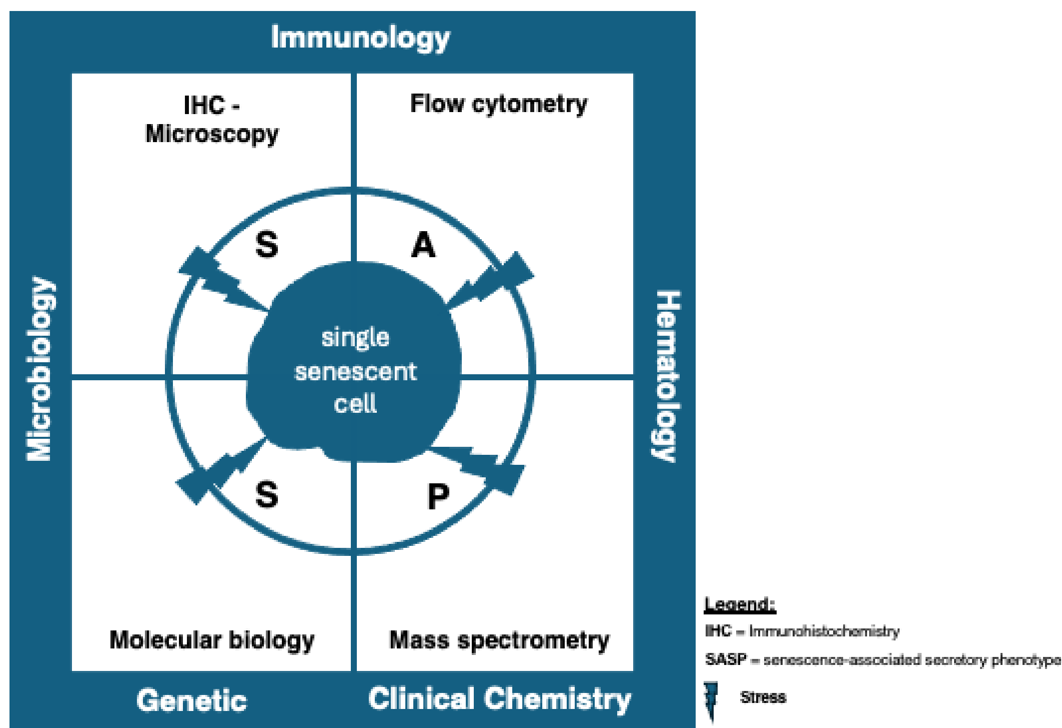

Figure 1 Methods for single-cell measurement of senescent cells. The arsenal of laboratory methods ranges from immunohistochemistry, flow cytometry, and routine medical laboratories to high-tech mass spectrometry and gene sequencing.

The focus is on localizing and quantitatively measuring these senescent cells at the single-cell level within the organism, utilizing direct surface and genetic markers [20, 31]. If genetic markers are identified in the organism under investigation, it is scientifically appropriate to compare them with a well-established marker atlas to facilitate accurate characterization and validation [32]. Through this, organ-specific aging of the patient can also be clarified using complex, new genetic methods. This knowledge can then be used for therapeutic purposes, including cellular improvement [33].

3.1 Immunohistochemistry – Microscopy

For the optical determination of senescent cells, immunofluorescence microscopy images are usually used for qualitative evaluation in addition to light microscopy images. Microscopy is mainly used by pathologists, hematopathologists, cytologists, and immunologists as a standard method, whereby many available staining techniques are used in histology and cytology to differentiate between normal or pathological cells, cell structures, tissue, and organs. In addition to the histological modification of cell and tissue structure, immunohistochemistry (IHC) is used to detect senescent cells [22]. The technique was invented at the beginning of the 1940s and is now a powerful microscopy-based method for visualizing cellular components, for instance, proteins or other macromolecules [34].

There are several available methods and markers: www.antibodypedia.com. The immunohistological examination spectrum depends on the disease and the specific clinical question. In addition, these classic staining methods can be used for the labeling of senescent single cells too. In aging research, organ-specific markers are combined with age-associated markers, such as anti-p53 antibodies, depending on the specific characteristics and requirements of the organ under investigation. Reference is made to standard cytology textbooks and established protocols, with this section focusing on a selected overview of immunohistochemical markers. The TUNEL method (TdT-mediated dUTP-biotin nick end labeling) in fluorescence microscopy is a molecular biology technique used to visualize the nuclei of apoptotic cells. However, challenges arise in distinguishing apoptotic cell death from necrotic cell death or mechanically damaged cells [35, 36].

A technically simple and frequently employed method for detecting cellular senescence is the immunohistochemical staining of cells with increased SA-β-Gal activity (SA-β-Gal, Senescence-associated beta-galactosidase) [37, 38]. This method enables the direct observation of senescent cells in tissue sections or cell culture and is, therefore, often used in oncology [39]. A special methodological feature of the lysosomal β-galactosidase, which accumulates in senescent cells, is its low pH optimum. Thus, performing the β-galactosidase activity assay at a pH of 6 enables the specific detection of senescent cells in vitro and in vivo. The test is named as SA-β-Gal assay [37, 40].

The SA-β-Gal assay is widely recognized and accepted as a marker of cellular senescence, but it presents a challenge in performing the measurements. Under certain experimental conditions, such as high cellular confluence in cell culture, pH sensitivity, or stress-induced conditions, differentiated or quiescent cells can lead to SA-β-Gal expression, resulting in false-positive results [41-44]. These limitations can be greatly reduced with a standardized measurement approach. This includes precise pH adjustment of the staining solution to an optimal pH value of 6.0 and the use of a CO_2-free incubator, as CO_2 can also lower the pH value. The fact that specific cells, such as macrophages, typically exhibit higher SA-β-Gal activity influences the measurement [45]. Laboratories should be aware of these limitations and should take them into account when assessing the results.

Another methodological characteristic of senescent cells is the accumulation of lipofuscin. Lipofuscin is an aggregate composed of oxidized proteins, lipids, oligosaccharides, and metals. Its presence is characteristic of aged tissue, is predominantly localized within lysosomes, and can be stained with a Sudan-Black B assay [46]. However, similar limitations in specificity as known from the SA-β-Gal assay are present and were, therefore, further methodologically optimized back in 2017 [47, 48].

3.2 Flow Cytometry

Numerous routine and research laboratories around the world use flow cytology (FACS, Fluorescence Activated Cell Sorting) for single-cell measurements as a quantitative, automated determination method. The great benefit of flow cytometry in hematology is undisputed, for instance, in the diagnosis of acute leukemia, multiple myeloma, myelodysplastic syndrome, or systemic mastocytosis. There is a large selection of CD surface markers (CD, Cluster of Differentiation) and the nomenclature of these markers is defined at the HLDA workshops (Human Leukocyte Differentiation Antigens) [49].

Depending on the combination of different staining markers, flow cytometry can be used in routine research to detect senescent cells [50]. In our view, this routine method is significant in

hematology and immunology due to its high level of standardization, which provides detailed information on the single-cell content for cellular analysis. Investigating differences in CD surface markers for an age-dependent immune profile in healthy individuals requires extensive studies using flow cytometry [51-54].

These studies show significant measurable differences in the immunological profiles of both adaptive and innate immune cells between young and old individuals. $CD8^+$ T cells decreased, and the CD4/CD8 (-) ratio increased, while NK cells (natural killer cells) also increased significantly. $CD4^+$ T cells were less affected by aging, while $CD8^+$ T cells significantly lost CD28 (-) and CD31 (-) expression with increasing age. The thymus reserve decreases at varying rates throughout an individual's life. Thymus reserve function can be quantified via the number of $CD31^+/CD4^+/CD45RA^+$ T cells in the blood [55, 56]. A clear reverse trend was observed in the naive and memory subsets of $CD4^+$ and $CD8^+$ T cells [53].

One interesting aspect is the connection between $CD8^+CD28^-$ old cells and less antibody production after immunization [57]. These $CD8^+CD28^-$ T cells are, therefore, defined as senescent T cells. Less than 50% of the $CD8^+$ T cell compartment of elderly or chronically infected individuals are $CD28^+$, while up to 80% of $CD4^+$ T cells retain their CD28 (-) expression even in centenarians [58, 59]. The downregulation of the costimulatory molecule CD28 is a hallmark of aging T cells. Increased $CD8^+CD28^-$ senescent populations with heterogeneous roles have been described in various solid and hematogenous tumors [60]. When characterizing naive T cells, a lower concentration of CD45RA-positive cells was defined as a “hallmark of aging” [61]. $CD45RA^+$ are naive T lymphocytes that have not yet had any antigen contact. The number of $CD45RO^+$ memory cells increases while the number of naive T lymphocytes ($CD45RA^+$) decreases [51, 52]. This leads to a poorer immune response, especially to “new” antigens [62].

Flow cytometry is particularly important in research on the aging immune system. Due to the progressive “immunosenescence” in older age (a term created by Roy Walford, 1924-2004), a clinic specializing in gerontology could concentrate on simple and inexpensive CD marker panels in the search for senescent T-cell changes. In addition to the large number of potential biomarkers, which are typically used indirectly, for example, the increase or decrease of a certain laboratory value in the blood or urine [21, 30], the CD4/CD8 ratio approach is very often used in flow cytometry for the aging immune system. This CD4/8 ratio is also used in the IRP, a cluster of immunological parameters, as one of the numerous biomarker combinations [21].

The CD4/CD8 T-cell ratio is an important marker for the development of many pathologies and for monitoring therapies. In 2021, Garrido-Rodriguez et. al. investigated immunological characteristics such as thymic performance and immune profiles for T cells at different CD4/CD8 ratio values, which define immune capacity and can influence the health status of older people. Thus, the CD4/CD8 ratio can be used as an integrative marker of biological age [63]. In our view, this is an excellent routine marker in FACS laboratory analysis.

Table 1 depicts a selection of CD markers and their target cells with link to cell ageing. Markers such as CD28, CD27, CD57 and the classic ratio formation of CD4/CD8 are usually used in routine diagnostics [64]. Here, the increase in CD57 and the CD4/CD8 ratio indicates an older cell population in the analysed blood. By investigating for suitable CD marker profiles for the study of human ageing, rare gamma/delta T cell subsets were also identified without finding clear markers in contrast to the alpha/beta T cells [65]. Several studies have shown that CD36 is highly expressed on the surface

of senescent cells, and CD36 plays a role in SASP production and regulation of lipid metabolism in senescent cells [33, 66, 67].

Table 1 Selection CD-marker for senescent cells (routine- & development-lab-level).

CD	Cell type	Laboratory	
Cluster of differentiation	Type of interest and functionality	Routine level	Development level
CD1c	Dendritic cells	Yes	Yes
CD3	Acquired immune cell	Yes	Yes
CD4	Acquired immune cell	Yes	Yes
CD8	Acquired immune cell	Yes	Yes
CD25	activate T-cells	Yes	Yes
CD27	Hypermutation & Isotype switch (in B-cells), Proliferation & Differentiation (in T-cells)	Yes	Yes
CD28	T-cells Coactivation of T-cells	Yes	Yes
CD31	Endothelial cells; a marker for the thymus reserve	Yes	Yes
CD36	platelet GPIV, CPIIIb, platelet adhesion molecule, involved in the recognition and phagocytosis of apoptotic cells. SASP regulation	Yes	Yes
CD45RO	Memory-T-cells	Yes	Yes
CD45RA	T-cell, naive T-Lymphocytes	Yes	Yes
CD51	Possibly a receptor for apoptotic cells	No	Yes
CD57	CD57 is a marker of terminal differentiation on human $CD8^+$ T cells	Yes	Yes
CD95	known as Apo-1, Fas, binds TNF-like FAS ligands, induces apoptosis	Yes	Yes
CD123	Innate cell	No	Yes
CD141	Innate cell	No	Yes
CD178	FasL, Fas ligand, binds to Fas to induce apoptosis	No	Yes
CD258	Triggers apoptosis?	No	Yes
CD261	Triggers apoptosis?	No	Yes
CD262	Triggers apoptosis?	No	Yes
CD358	Triggers apoptosis?	No	Yes
CD366	Triggers apoptosis?	No	Yes

Legend: CD: Cluster of Differentiation; SASP: senescence-associated secretory phenotype; FasL: Fas-Ligand, Apo-1: Apolipoprotein; TNF: Tumour necrose factor.

CD markers are not universally reliable for identifying senescence and are generally used alongside other assays, such as SA-β-Gal staining, DNA damage markers (e.g., γ-H2AX), or SASP profiling, to confirm the senescent phenotype [37, 40, 68-70]. For an exact quantitative evaluation of senescent cells using FACS, a counterstaining on SA-β-Gal is often used in routine to the CD

markers. A modified SA-β-Gal method is employed here and enables the quantitative determination of living senescent cells, but with the disadvantage that these cells must be released from the cell or tissue network [40]. Here, the enzymatic activity of the lysosomal SA-β-Gal is measured using its substrate, C12FDG (5-dodecanoylaminofluorescein-di-β-galactopyranoside) [40]. For example, it has been shown that senescence-associated β-galactosidase indicates the frequency of senescent $CD8^+$ T cells in ageing humans [71].

However, the limitations of the use of the SA-β-Gal assay, as described in Chapter 3.1, must also be taken into account when practicing it in flow cytometry [41-45]. The benefit of combining CD markers with γ-H2AX [72] due to its accumulation in the cells as a damage marker or of p53, p21 and/or p16 with increased levels of cell cycle regulators, leads to an improvement in sensitivity and specificity for the detection of senescent cell type in routine and research [33]. To further increase the quantitative identification of senescent cells, combinations of these supporting assays can also be used, such as a combination of SA-β-Gal and γ-H2AX [42]. A selection of additional supporting biomarkers for senescent cells in FACS analysis is shown in Table 2.

Table 2 Selection of additional supporting biomarkers for senescent cells in FACS analysis (routine- & development-lab-level).

Name	**Information**	**Laboratory**	
Fluorescent labelling Antibodies	Type of interest and functionality	Routine level**	Development level
SA-β-Gal	Production/Accumulation (Overexpression Lysosomes, C12FDG – Signal)	Yes	Yes
γ-H2AX	DNA damage	Yes	Yes
p53	Cell cycle arrest	Yes	Yes
p16	Cell cycle arrest (P16INK4a expression)	Yes	Yes
p21	Cell cycle blockade	Yes	Yes
IFN-γ	SASP, Inflammation	Yes	Yes
IL-6*	SASP, Inflammation	Yes	Yes
IL-8*	SASP, Inflammation	Yes	Yes
TNF	SASP, Inflammation	Yes	Yes

Legend:

Routine level** – Here we mean special laboratories with a focus on gerontology.

SA-β-Gal – Senescence-associated beta-galactosidase.

SASP – Senescence-associated secretory phenotype.

TNF – Tumor necrosis factor.

IL* – Interleukin.

IFN – Interferon.

Note: For the detection of SASP products such as IL-8, IL-6 or IFN gamma, which are excreted in the culture medium, other methods such as ELISA can also be used.

Senescent cells have a typically larger morphology than cycling cells; forward scatter and side scatter parameters can be used in the gating strategy to separate the population in senescent cells

with weakly upregulated SA-β-Gal expression [68]. The evaluation programs for flow cytometry are constantly being improved by the manufacturers [42, 73, 74]. The use of artificial intelligence (AI) Amnis and machine learning (ML) tools IDEAS are helpful for optimization of the applications of methods for measuring – for instance, the classical senescence marker like the SA-β-Gal assay [75]. This will further support FACS analysis of senescent single cells in clinical areas such as radiology and oncology [74].

Recent advancements in artificial intelligence and deep learning have introduced nuclear morphology as a reliable and noninvasive biomarker for identifying cellular senescence [76]. The researchers showed that morphological changes in the cell nucleus can serve as a deep learning predictor for senescence. These recent advances in the field of artificial intelligence and deep learning are applicable across tissues and species (mice, human dermal fibroblasts) and are also linked to health outcomes in humans. A neural network classifier trained on fibroblasts maintained in cell culture on DAPI-stained nuclei with background removed. Human dermis shows an increase in DAPI staining in senescent nuclei with age. By training additional models on samples with reduced features, the researcher showed that the shape of the nucleus alone provides a signal to indicate a senescent state [76]. Due to the complex structure of the cell nuclei, the researchers used Xception, a powerful model for image classification in biomedicine [77, 78].

Once a specialized laboratory has established the appropriate CD marker panel for its patient collective on the FACS, AI (Artificial intelligence) evaluations of the generated databases of the individual measurements are required in order to compare them with the clinical data and the often additionally generated laboratory data from other disciplines or a self-defined evaluation set in order to be able to classify the CD markers in a meaningful way [20]. A common approach in the laboratory here is to focus on lymphocyte cells that can be easily measured in the routine using FACS. This focus on a key element also facilitates the overview of the patient's medical report (see Chapter 4).

The extensive use of flow cytometry offers the potential to obtain a quick overview of the cellular status - down to the single-cell level - of patients, even in routine practice. In our opinion, the use of the CD4/CD8 ratio and the determination of CD28/CD8 has proven its worth here. As described in Chapter 5, we also recommend flow cytometry as an ideal routine method for clarifying cellular immunodeficiencies and as a successful control after treatment with senolytics or senostatics in any form.

3.3 Genetic Methods

Almost five decades of sequencing and the technologies continue to develop and advance. Since DNA sequencing was first possible in 1975 due to the methodical concept of dideoxy sequencing or Sanger-sequencing, shot-gun-sequencing, and even the cloning of larger genomes improved the method in the following years [79, 80]. The first successful and almost complete sequenced genomes were those of model organisms such as *Saccharomyces cerevisae, C. elegans* and *Drosophila melanogaster*, applying a combination of Sanger- and shot-gun-sequencing [81-83]. The whole-genome random shotgun sequencing (WGS) became a very effective method and was the key technique applied in the international Human Genome Project (HGP), see Figure 2 [84, 85].

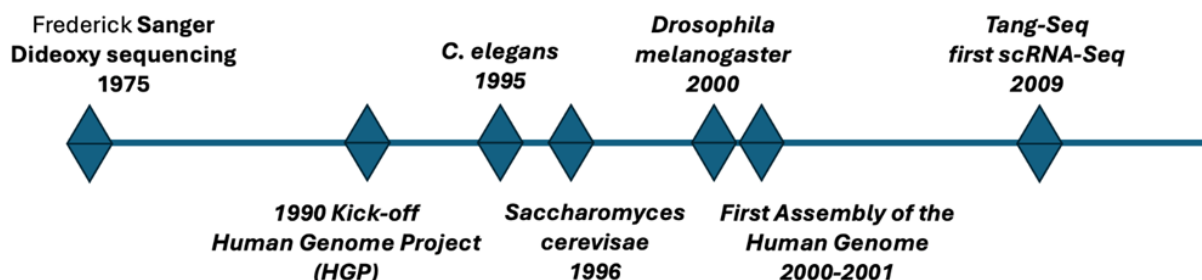

Figure 2 A journey through DNA decoding.

The beginning of this revolutionary scientific milestone was in 1990 after almost five years of preparation time. First presented back in 1985, the idea was met with mixed scientific response at the time. Nevertheless, over the course of the project, the HGP established two assembly approaches, which enabled the international consortium to complete an initial draft assembly of the human genome reads in October 2000. Remarkably, the actual sequencing process itself took only nine months. The results were further improved and finished in 2001 [86, 87].

With the development of massively scalable, parallel, and throughput sequencing devices (e.g., Pyrosequencing, Ion Torrent, Illumina/Solexa, and SOLiD), a new era was heralded [88-90]. These next-generation sequencing platforms modernized DNA sequencing with partially different strategies and reduced costs rapidly. Sequencing by synthesis (SBS) is based on the principle of enzymatic DNA synthesis, where new nucleotides are detected in real-time. Some SBS technologies utilize on-surface, in-situ amplification (e.g., Illumina), which is primarily a “short-read” technology. Others enable single molecule sequencing (SMS), allowing the direct detection of each nucleotide from an individual DNA strand, typically generating “long-reads” (e.g., Oxford Nanopore Sequencing or Pacific Biosciences) [91-93]. Nanopore is currently the sole technology that enables, besides DNA and cDNA also, the unbiased, direct sequencing of native RNA without requiring upstream reverse transcription or amplification. This makes it very suitable for addressing modification-related questions [94-96]. Recent advancements in nanopore technology, using the ClpX motor protein, enabled direct protein sequencing by guiding the protein strands in a two-step process through a CsgG pore for single-molecule analysis of the amino acid sequences [97].

Single-cell- and single-nucleus RNA sequencing (scRNA-/snRNA-seq) allow the registration of genetic information of cells individually and profiling of their actual diversity. Whereas bulk RNA sequencing simply mediates only an overview of total cells [98-100]. The initial step involves the isolation and separation of cells, a critical process that is fundamental for downstream applications. Common methods for cell-sorting include: FACS, Magnetic-Activated Cell Sorting (MACS), Micro-fluidics/droplets-based (InDrop/Drop-seq, 10× Genomics/Chromium, Parse Biosciences), and Nanowell-based approaches (BD-Rhapsody) [101-106]. One of the first developed methods for single-cell whole transcriptome was introduced in 2009. Tang-seq is a pioneering method for single-cell mRNA sequencing that enables whole-transcriptome analysis by amplifying and sequencing the mRNA of an individual cell, providing insights into gene expression at single-cell resolution. The incorporation of a unique barcoding strategy allowed the efficient capture and identification of the RNA out of the single cells. This minimizes amplification bias and improves the accuracy [107]. As a general principle, it is crucial to choose a sc/snRNA seq technique that has the strongest performance and aligns with the specific need to answer scientific or clinical questions [108, 109].

In the context of aging, Human PBMC scRNA-seq (Illumina HiSeq) based aging clocks reveal the balance between ribosome activity and inflammation as a key hallmark of cellular aging and a potential marker for super-longevity [110]. Epigenetic age prediction utilizes DNA methylation patterns to estimate biological age, its mechanisms and age-related diseases [111]. The “PromethION” and “GridION” sequencing systems developed by Oxford Nanopore Technologies (ONT) hold significant potential, power, and deeper insight for advancing research in single-cell transcriptomics, as well as in age- and cancer-related studies [112-115].

High throughput single-cell long-read sequencing can reveal the phenotypes of human tumors [116, 117]. Nevertheless, even the smallest device “MinION” offered by ONT, can detect mutations in the *TP53* gene, which encodes for p53 [118-120]. Additionally, mitochondrial DNA (mtDNA) deletion and or mutations that are associated with age-induced mitochondrial dysfunctions or diseases were much more frequent and expanded identified by long-read sequencing [121]. Telomere biology and length are highly related to aging and cancer. Telo-seq employed on ONT platforms offers a high resolution that provides insight into the dynamic changes in telomere length and structure [112, 122].

3.4 Mass Spectrometry

MALDI-TOF mass spectrometry (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) for single-cell analysis of senescent cells (with single-cell resolution) is not yet sufficiently established in the routine of medical laboratories. In contrast, MALDI-TOF is already fully established and indispensable in microbiology laboratories. A great deal of development work is still required to obtain an easy-to-use and cost-effective instrument for the determination of labeled “single cells” for routine use in gerontology. In contrast, the Exent® system from Binding Site for the diagnosis of monoclonal gammopathy in Switzerland could soon go into routine use much more quickly. EXENT® Solution (Thermo Fisher Group) is a fully integrated and automated mass spectrometry system designed to transform diagnosis and assessment for patients with monoclonal gammopathies, including multiple myeloma. The advantage of this new technology for modern precision medicine in a routine laboratory for the detection of very few malignant cells is clinically validated. However, the higher laboratory costs associated with this highly sensitive single-cell measurement technique, along with unresolved insurance-related issues regarding reimbursement compared to standard methods like immunofixation for detecting monoclonal gammopathy, are hindering a rapid market launch of this important diagnostic tool for tumors. So far, recommendations from the International Myeloma Working Group (IMWG) Mass Spectrometry Committee are only available for tumor monitoring [123, 124].

The Mayo Clinic group is working on the investigation of monoclonal gammopathy using an assay developed in-house on the MALDI-MS system [123]. Due to the high sensitivity for the detection of single cells in the context of senescence clarifications, this advanced technique is used as a method in senescence research [125, 126]. Studies using the MALDI and CyTOF methods on senescent mesenchymal cells in vivo to identify specific skeletal cell populations have shown that these can be eliminated by senolytics [126]. Bringing these types of mass spectrometry systems to a routine application level for identifying senescent single cells in aging research is currently met with skepticism due to the high associated costs.

3.5 Notes and Brief Overview of Limitations, Reliability, Accessibility and Costs of the Methods

To obtain the best result for single-cell measurement, the choice of method depends on the clinical/scientific question and the limits of the method in terms of clinical applicability. In the microscopic diagnosis of tumors, malignant cells are distinguished from normal cells using immunohistochemistry. Mass spectrometry can then use the protein pattern created to provide an indication of the aggressiveness of these cells and their sensitivity to therapy. The absolute benefit of flow cytometry is to provide a quantitative, exact cell count of the examined blood with simultaneously analyzed CD, senescence and/or leukemia markers. The sequencing of these cells in the molecular biology laboratory is the genetic method. Thus, each available method has its strengths around the application of the question.

Regarding the detection limits of the methods described in Chapter 3, the focus is placed on mass spectrometry and flow cytometry. The mass spectrometry method, e.g. with the Exent® system, can detect monoclonal paraproteins (in a 0.2 g/L polyclonal background) from approx. 0.015 g/l [127]. This sensitivity aids in the detection of M-proteins below the levels of conventional methods [128]. The specificity for monitoring M proteins based on their unique molecular mass is a precision of ±4 m/z (Manufacturer device information: The Binding Site part of Thermo Fisher Scientific; Immunoglobulin Isotypes (GAM) for the EXENT® Analyzer Instructions for Use, August 2024 PBR005_0824). Gammopathy diagnostics using mass spectrometry is approximately 10 times more sensitive than the traditional serum-based methods of serum electrophoresis and immunofixation [129]. With standard mass spectrometry, 200,000 cells are usually sufficient to create a "normal" total proteome profile, e.g. for human cell lines, which corresponds to around 20 µg of total protein. However, the required number of cells can vary greatly depending on the size of the cells.

Multiparametric flow cytometry (MFC) shows high sensitivity (down to $1:10^{-3}/10^{-5}$ cells) and is mostly used for MRD quantification (MRD, measurable residual disease) due to its routine suitability with a high degree of standardization. The identification of 20 clustered leukemic residual cells is established for the detection of the presence of MRD (LOD, lower limit of detection), while a cluster of 50 events can be the minimum threshold for the quantification of a cell population (LOQ, lower limit of quantification). The prerequisite is that a sufficient denominator of relevant events (500,000-1,000,000) is recorded [130]. It is generally stated in flow cytometry that 20 events can be assumed for the smallest (homogeneous) population that can be recognized in a list mode data file by experienced operators. This is also one of the reasons why flow cytometry has a high degree of reliability in the quantitative detection of senescent cells in aging research.

However, the reason for the limitations in the clinical application of the various methods often lies not only in the device or the method itself but also in the pre-analysis, as poor sampling or incorrect sample preparation can interfere with or block the selected measurement method. For example, cell changes can occur in flow cytometry if the transport time is too long, exceeding 24 hours at room temperature, in a blood sample obtained by venipuncture. These changes influence and falsify the result. A biopsy performed at the wrong location also leads to an incorrect result with the methods discussed. The reliability and limitations of mass spectrometry for the detection of protein patterns in individual cells can also depend heavily on the available comparative databases. If there is no comparative data, the measurement cannot be assigned during evaluation.

The benefits of single-cell measurement of senescent cells in precision medicine are undisputed. The accessibility of the various methods should, therefore, be ensured for specialized physicians,

gerontologists, and aging researchers depending on the specific clinical or scientific question. Another important point here is the availability of the method for urgent clinical questions.

Immunohistochemistry has good and practical accessibility for medical doctors and researchers for many questions. Thanks to decades of experience with this historically oldest method, the laboratory work involved is easy to handle and technically manageable. However, the accessibility of mass spectrometry for single-cell measurements is less good. Currently, single-cell measurements of senescent cells using mass spectrometry are mainly carried out in clinical trials at university hospitals, usually with an affiliated research laboratory. Accessibility to the topic of single-cell measurement by mass spectrometry is therefore primarily available to clinicians and researchers on-site at a university hospital or research institution. However, the authors would like to underline at this point that mass spectrometry is also part of routine analysis in private medical laboratories in the departments of microbiology and clinical chemistry. A major advantage of mass spectrometry is the rapid analysis time for patients.

Flow cytometry is established in university hospitals, larger hematology centers and private laboratories with a focus on hematology and is therefore highly accessible to general practitioners, gerontologists and other specialists. The fact that aging research institutes at universities are often equipped with a flow cytometer for research purposes also increases the accessibility of this method. An important advantage of this quantitative measurement method for clinical follow-up is also the availability of ready-to-use, standardized CD marker panels, which can be easily used depending on the clinical question. The respective manufacturers also supply the corresponding gating settings with evaluation software for flow cytometry. This method is permanently standardized by EuroFlow [131].

When we talk about and critically evaluate the laboratory costs of the methods presented above in 2025, we can currently only give very basic indications of the prices of the devices. The indicated price ranges are subject to large fluctuations and are to be regarded as the lowest acquisition costs for the basic equipment of the respective device or for performing a specific method. It should be noted that no standard laboratory equipment, e.g., Laboratory benches, freezers, pipettes, or laminar-flow workbenches, are, not included. Cost information is of interest to the user for practical reasons and is also important to provide guidance when planning to purchase an analyzer based on a predetermined specification or when a new method is to be introduced. Prices are also a matter of negotiation and are subject to the respective market conditions. When comparing individual methods with one another, it should be noted that the costs for the maximum equipment of a system with, e.g.: Evaluation software with the use of AI, pipetting robots, autosamplers, etc. can be far higher - i.e. the higher the quality of the technical equipment and software, the higher the price. To be able to make a cost comparison of the various methods, we have the following information from daily practice:

A laboratory can expect to pay around €100,000 to €200,000 for a good standard flow cytometry system and around €200,000 to €400,000 for a mass spectrometry system. Particularly in the case of specialized mass spectrometry systems (MALDI-MS, EXENT®), the acquisition costs, together with the necessary databases and special pipetting and software solutions, tend to be in the upper range, but also higher. One disadvantage of mass spectrometry is, therefore, the high acquisition costs and the high costs of the databases. However, this disadvantage must be weighed up against the superiority of the modern measurement method in terms of higher sensitivity and specificity compared to established laboratory methods (e.g. Exent® versus immunofixation in serum). The

regular follow-up costs for servicing the devices that are necessary for operation are also fundamentally important for calculating the profitability of a laboratory device. Here, an average of approximately 10% of the new purchase price of a system per year must be taken into account.

The average acquisition costs for the introduction of standard immunohistochemistry are roughly between €100,000 and €150,000 for the basic equipment with, for example: a simple staining machine and a microtome for tissue sections and manual evaluation on the microscope plus low-cost management software. The detection of proteins in intact, formalin-fixed, paraffin-embedded tissue is quantitative here. Besides, the cost of upgrading this method and updating the technical equipment, especially when working with the support of AI software in pathology, can be expected to be considerably higher too.

A molecular biology laboratory has also to deal with initial acquisition costs of around €100,000 to €150,000. The price ranges for standard devices are, for example, €5,000 - €25,000 for a PCR thermocycler, €15,000 - €40,000 for a real-time PCR (qPCR) and €5,000 - €20,000 for a DNA extraction device (e.g. EasyMag™) in order to be able to use a very sensitive and highly specific method for single-cell detection in the laboratory. In this case, the cost limits are open as well, depending on the research focus or clinical application and license. As far as the feasibility of implementing single-cell measurement in a clinical setting is concerned, we see single-cell analysis as preferable in larger medical centers, such as university hospitals with affiliated research laboratories, due to the high costs and the need for scientific staff.

4. Integrated Laboratory Reports in Gerontology

Nature always attempts to achieve a state of pleiotropy - homeostasis between young tissue and old tissue in the organism. The medical treatment also aims to bring the patient back to a healthy condition. Laboratory values give conclusions about our health condition, thus the “laboratory status” of the patient. In a classic medical routine laboratory preferentially, inexpensive established analyses are used for screening. Medical doctors select out of the entire range of standard laboratory tests and medical analyses, e.g., ELISA (enzyme-linked immunosorbent assay), and EIA (enzyme immunoassay). The measured values, derived from the patient's body fluids, together provide a comprehensive view of the “total senescence” at the laboratory level. All analysis values measured from the patient’s material sample (blood, urine, saliva, and others) are listed in a report, depending on the laboratory’s specialty. Private medical laboratories and state laboratories specialising in gerontology offer a variety of senescence assessments as part of their diagnostic profiles from the patients.

In most cases, both the doctor and patient receive a standard laboratory report or a specialized age report, depending on the analysis profile order. A key limitation in this context is often the absence of important clinical information about the patient in a structured format, such as medication details and, where available, the patient's age-related status from a prior geriatric assessment, such as a frailty index. In today’s available laboratory findings, organ-specific analyses are employed for “organ-ageing” in the context of functional loss in older age, e.g. Creatinine-Kinase for the kidneys or ALAT (alanine aminotransferase) for the liver. In healthy individuals, laboratory values can be compared with age-dependent reference ranges established from a cohort of older healthy donors, providing an indirect indicator of biological age [132, 133].

There is evidence that there is less T-cell maturation starting at the age of 50. Both the quantity and quality of T and B cell responses change, which has a negative impact on the effectiveness of the immune response in old age [19, 62]. This is another reason why we become more susceptible to infections in old age and react less well to vaccinations. This also contributes to the development of age-related diseases and cancer [62]. The gender- and age-specific reference ranges of CD markers for peripheral immune cells can serve as a basis for effective immune monitoring using flow cytometry (FACS) in age-related diseases [53]. This crucial information should also be practically applied in assessing immunity in older adults by flow cytometry. In the laboratory, a standard cellular analysis to assess cellular immunodeficiency in older adults can be efficiently performed by quantifying lymphocyte subpopulations in EDTA blood. However, an isolated list of individual measurement results for cell surface markers (CD markers) in a laboratory report often provides relevant information only to specialists, making it difficult for others to interpret.

The idea for optimized laboratory analysis in gerontology is analogous to the integrated CSF (cerebrospinal fluid) reports in neurology. The “integrated cerebrospinal fluid report” with all relevant tests from all medical laboratory areas (immunology, microbiology, clinical chemistry, hematology, genetic) are often already routine in neurology [134] and, in the authors' view, should also gain more importance in the field of gerontology for the assessment of the patient's senescence status in a medical laboratory.

An integrated laboratory report in gerontology, which combines measured laboratory data with clinical observations into a clear and concise "integrated aging report," is still rarely available in many cases today. A key challenge lies in the manual effort required to integrate existing data into the laboratory information system (LIS). This transfer of clinical data frequently relies on manual input into the computer by healthcare professionals. The report (DIN A4) should then be provided to a medical doctor, that consolidates all essential laboratory values and clinical details, offering a quick and comprehensive view of the patient’s senescence status.

However, a general problem is that there are fewer age-relevant reference range values available. To possibly counteract the problem, a better age-appropriate reference range should be implemented to assess the current measured values, depending on the patient’s population. It is inappropriate to compare patients aged 60 years with reference values established for younger populations, such as 30-year-old adults. Age-specific reference values, for example for CD surface markers or for the platelet count, can provide clues for an age-specific assessment of the measured single cells [53, 54, 135].

The authors believe that such reference value studies should be intensified in the future, especially due to the aging of the Western population. However, in most cases, the timing of clarification of CD surface markers often does not reflect the chronological age but is rather related to the biological age. Therefore, it seems crucial to include age-dependent reference values in the assessment of an overall report.

Clinicians and general practitioners derive the greatest practical benefit when laboratory results are presented in a more compact and optimized format, enabling them to quickly assess the patient's initial aging status within the constraints of their limited consultation time. The summary on one page with the most important information would be particularly beneficial. Furthermore, laboratory values, with an associated organ status and, depending on the clinical manifestation such as an existing disease, could also be presented and interpreted in the overall view of the patient. The laboratory reports in gerontology, in connection with the patient's medical history and the

associated clinical assessment, could include the following points and analyses suggested in Table 3. The specialized gerontological institute or laboratory puts together the relevant analyses into an integrated gerontology report, either for internal use or customized to the specific clinical question.

Table 3 Selection of analyses and recommended components of the integrated laboratory report in gerontology "Senescence report that fits on one page".

Gerontology "Senescence report"				
1.	**Clinical Assessment**			
	- Medical history			
	- Medication history			
2.	**Patient Classification**			
	- Fragility score (if available)			
3.	**Laboratory Results**			
	- Reference values (age dependent reference values)			
	- Personalized reference values (the patient's individual reference values)			
	- Age-appropriate selection of analyses (see the specialized categories below)			
4.	**Clinical Chemistry**	**Hematology**	**Immunology**	**Senescent single cell**
	hsCRP*	CBC*	IL-1*	CD4/8* ratio
	HbA1c*		IL-6*	CD8/CD28*
	ALAT*			Lymphocyte typing
	Creatine kinase			
	Ferritin			
5.	**Organ-Specific Analysis ("Organ status")**			
	- **Liver:** ALAT*, AP*, hsCRP*			
	- **Kidney:** Urine acid, Creatine kinase			
	- **CNS:** Dementia markers (only if suspicion of dementia)			
6.	**Additional Analyses** (Further organ-specific marker analyses, if necessary)			

* **hsCRP** = High-sensitive C-reactive protein.
ALAT = Alanine Aminotransferase.
AP = Alkaline phosphatase.
HbA1c = Glycated haemoglobin.
CBC = Complete blood count.
IL = Interleukin.
CD = Cluster of differentiation.

This integrated report can also include a link that directs users to specific marker analysis results and protocols, providing additional information as needed. As part of this, the general practitioner, geriatrician, and gerontologist should receive a concise overview of the patient based on clear key pieces of information from the report. The aging process, senescence, is a complex process, and therefore, a strong examination system is needed, which may be possibly processed by Artificial Intelligence (AI) in the future. This high-tech medicine may provide a link to conclusions on individual topics, which could be entered as questions by the examining doctor and should be

considered. To sum up, single-cell analysis in combination with routine analyses from the medical laboratory can thus be practically applied to the patient.

5. Discussion - Benefits and Outlook of Single-Cell Analysis for the Future

The biological process of aging is partly attributed to the accumulation of senescent cells in the organs. Detection and treating senescent single cells not only carry therapeutic potential but also offers the possibility of “repairing or restoring the aged cell status”. To address the task of slowing rapid aging or influencing the aging state (possibly even achieving an improvement) a standardized and structured detection for senescent cells in the organism is necessary. Detecting these senescent cells requires appropriate targeting and the selection of a suitable detection method (see Chapter 3).

The targeted labeling of senescent cells also provides the basis for therapeutic options using, for example, neutralizing antibodies or nanoparticles [33]. SA-β-Gal staining is one way of detecting aged single cells in oncology [39]. The targeted removal of senescent cells after labeling is employed to inhibit tumor growth and positively impact the course of therapy. However, the direct identification of senescent cells in vivo in humans still represents a major methodological challenge [1]. Further research is needed to develop imaging techniques to identify localized accumulations of senescent cells in clinical trials with senolytics and to follow their development [136]. Senescent cells are involved in processes characterized by increased tissue formation and restructuring, such as embryogenesis, and in connection with regenerative processes, such as wound healing. Thus, the selective and effective removal of senescent cells from different cell types must be carried out in a very controlled and careful manner [137].

In mouse experiments for selective senescent cell removal, a prodrug strategy was used to develop a new drug based on the increased activity of lysosomal β-galactosidase (β-gal), a hallmark of senescent cells [4]. The strategic approach to influencing and re-modulating the SASP environment with therapeutics also requires the measurement and localization of labeled senescent cells [25]. This allows the SASP area to be narrowed down and to record the effectiveness of potentially available therapies against age-related diseases [138, 139]. Nowadays, the drug class of “senolytics”, targeting senescent cells, and the drug class of “senomorphics”, promoting healthier aging, are available as options for modulating the senescence-associated secretory phenotype (SASP) [136, 140, 141].

To therapeutically address age-related diseases using senolytics and senomorphics, it is first necessary to gain a detailed overview of the relevant cellular structures down to the single-cell level of the patient [141]. Many research and therapeutic approaches for senescent single cells or tumor cells often follow an organ-specific pathway. Therefore, the use of these senotherapeutics was typically tested for diseases affecting a single organ. However, their greatest strength lies in the potential to treat multiple age-related diseases simultaneously [142].

The desire to rejuvenate one's body and its aged cells, effectively reversing the effects of aging by several years, requires the application of various advanced medical treatment methods [44, 138, 140, 143-145]. Because of the accumulation of senescent cells in old age and the fact that senescent cells are also found in the context of age-related diseases (atherosclerosis, Alzheimer's, pulmonary fibrosis, osteoarthritis), the attempt to reduce these cells would be beneficial. The application of suitable analytical methods to first detect and then restore senescent cells is an ongoing challenge

[139, 140]. Autologous blood donation after treatment against senescence or “rejuvenation of senescent cells” would be an additional possible field of application for this not entirely cheap routine analysis. In principle, the concept of "cell restoration" has been familiar for quite some time, evident in practices such as bloodletting and, more specifically, in the physical optimization of competitive athletes through autologous blood transfusions [146].

The refunding of autologous blood or blood from a donation collection is very useful for planned operations [147]. Good therapy concepts have also been available for decades in the fight against tumor cells; see the book Therapeutic Hemapheresis in the 1990s in the chapter “Ex vivo activation and retransfusion of white blood cells” – [148]. Plasmapheresis or therapeutic hemapheresis, a method of removing harmful proteins, has become a proven routine method [148] in addition to the curative application of this mechanically optimized therapy method (e.g., for GBS, Guillain-Barré syndrome), a further area of application could be in the field of “technical senescence stabilization” to maintain a stable cellular status for as long as possible. The mechanical elimination of individually labeled senescent blood cells by performing hemapheresis using Cell sorting MaxTechnology is well established. The selective removal of senescent cells is a therapeutic option to reduce their negative immunomodulatory effects. The corresponding depletion of senescent cells in oncology is already applied, as mentioned above. An approach to improve fibroblasts and smooth muscle cells by removing chronically senescent cells, which named the authors "mid-old cells", reduced low-grade inflammation and improved tissue repair capacity [2].

However, the “Therapeutic hemapheresis for anti-aging” system must be applied very carefully [149, 150]. For instance, certain older immune defense cells (memory cells) need to remain in the patient's blood. Blood washing for rejuvenation using magnetic beads - efficient sorting of senescent cells [68] or a type of optimized autologous blood donation for rejuvenation will probably only continue to be available for a small number of wealthy patients in the future. A very promising approach to rejuvenating the immune system has already been demonstrated in mice. If young stem cells were implanted into the mice, they showed a phenotypically and functionally young immune system, comparable to the immune system of a young mouse [151]. In comparison, the mice that had received old stem cells showed an old immune system that was like the immune system of older animals. In addition, these mice responded less well to immunization. The researchers were able to demonstrate the rejuvenation of the immune system with the help of young stem cells using flow cytometry and are now trying to transfer this approach to humans. The rejuvenation of hematopoietic stem cells was also observed using the substance CASIN (Cdc42 activity-specific inhibitor) [152].

6. Conclusions

In today's era of precision medicine, the available methods for single-cell analysis are increasingly essential for therapeutic interventions targeting aging and age-related diseases. Effective senotherapy —the targeted modulation of senescent cells to improve aging or, alternatively, to delay natural aging and associated diseases—would be inconceivable without these advanced measurement techniques. Modern approaches for single-cell analysis of senescent cells, such as immunohistochemistry, flow cytometry, genetic detection methods (NGS), and mass spectrometry, promise significant advancements in knowledge, paving the way for new therapeutic options in gerontology. The introduction of a dedicated and integrated "gerontology report" could further

enhance laboratory services for clinicians in the future. At the same time, it raises critical questions about whether these single-cell measurement methods should also be applied in lifestyle medicine. Nevertheless, the primary focus of these technologies will remain on assessing the success of therapeutic interventions in gerontology and oncology.

To keep this field of precision medicine on track and aligned with public health cost considerations, further research, and methodological advancements are necessary. These include improvements in cell targeting, measurement techniques, and imaging technologies in human medicine.

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Author Contributions

Both authors worked equally on the review. Stefan Hardy Lung with a focus on molecular biology and Thomas Lung with a focus on flow cytometry.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

This review includes no data.

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