Santander X Innovation Xperts

Targeting cellular senescence to deconstruct aging

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By Santander

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Corina received an M.D. from Universidad Complutense de Madrid in Spain in 2017. During her medical studies she also performed extensive research work at the Spanish National Cancer Research Center, Harvard Medical School, the University of Oxford and the European Molecular Biology Laboratory. Upon graduation, she enrolled in the Gerstner Sloan Kettering Graduate School at Memorial Sloan Kettering Cancer Center in NY where she earned a Ph.D conducting her doctoral research in the laboratory of Scott W. Lowe. Her thesis work focused on developing immune-based strategies to target senescent cells. This included studies to identify surface markers of senescent cells, pioneering the use of CAR T cells as senolytics and the development of flexible somatic cancer mouse models. Her work on senolytic CAR T cells was widely highlighted in leading journals in the field and earned her the Chairman's Prize Award from Memorial Sloan Kettering. In 2022 Corina established her own research group at Cold Spring Harbor Laboratory whose goal is to study cellular senescence in cancer and aging and the development of cell-based senolytics.

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Introduction

Gaining a deeper understanding of aging biology is a pressing health and socioeconomic issue. In this regard, senescence is a key, but poorly understood determinant of organismal aging.

Cellular senescence is a stress response process whereby cells stop dividing and performing their function and instead become highly proinflammatory entities. In physiological conditions, this inflammation recruits immune cells which in turn eliminate the senescent cells and restore tissue homeostasis. Thus, while senescence is beneficial in younger organisms, the process is dysregulated over time because of aging of the immune system itself leading to a chronic inflammatory microenvironment that is key to aging and age-related pathologies.

Genetic animal studies have shown the therapeutic potential of eliminating senescent cells to extend both healthspan and lifespan. Translating these strategies to therapeutic avenues that could be used in the clinic in the short future, however, is still an area of ongoing investigation.

Here we discuss the evidence of the role of senescent cells in aging pathology, biomarkers for their identification and explore **current and potential therapeutic avenues for their elimination.** Overall, tailored strategies that eliminate senescent cells in specific pathologies harbor the higher efficacy and safety potential.

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Relevance of aging research

Currently, the percentage of individuals over 65 years old outnumbers the percentage of children under 5[1] and by 2050 the United Nations projects that it will also outnumber the proportion of people aged 15 to 24 years old[1,2]. Despite these demographic changes, healthspan, the part of a person's life during which they are generally in good health, however, has remained more constant[3] and there has been an increase in chronic "age-related diseases" such as vascular disease, diabetes, cancer and dementias[3]. Importantly, previous studies have shown that the **biggest health and economic impact will result from targeting the aging process itself rather than any individual disease**[3], highlighting the need to understand aging biology.

To date, nine hallmarks (or molecular mechanisms) of the aging process have been described, which can be grouped into three major categories: primary hallmarks or cause of damage, antagonist hallmarks or responses to damage and integrative hallmarks or manifestations of the aging phenotype. To be considered a hallmark a cellular process needs to fulfill 3 criteria[4]:

1) be present during physiological aging;

2) its aggravation should accelerate aging and

3) its elimination has to improve the pace of aging. Cellular senescence fulfills all three criteria with genetic studies showing a direct improvement in both healthspan and lifespan by the elimination of senescent cells[5,6].

03

Cellular Senescence

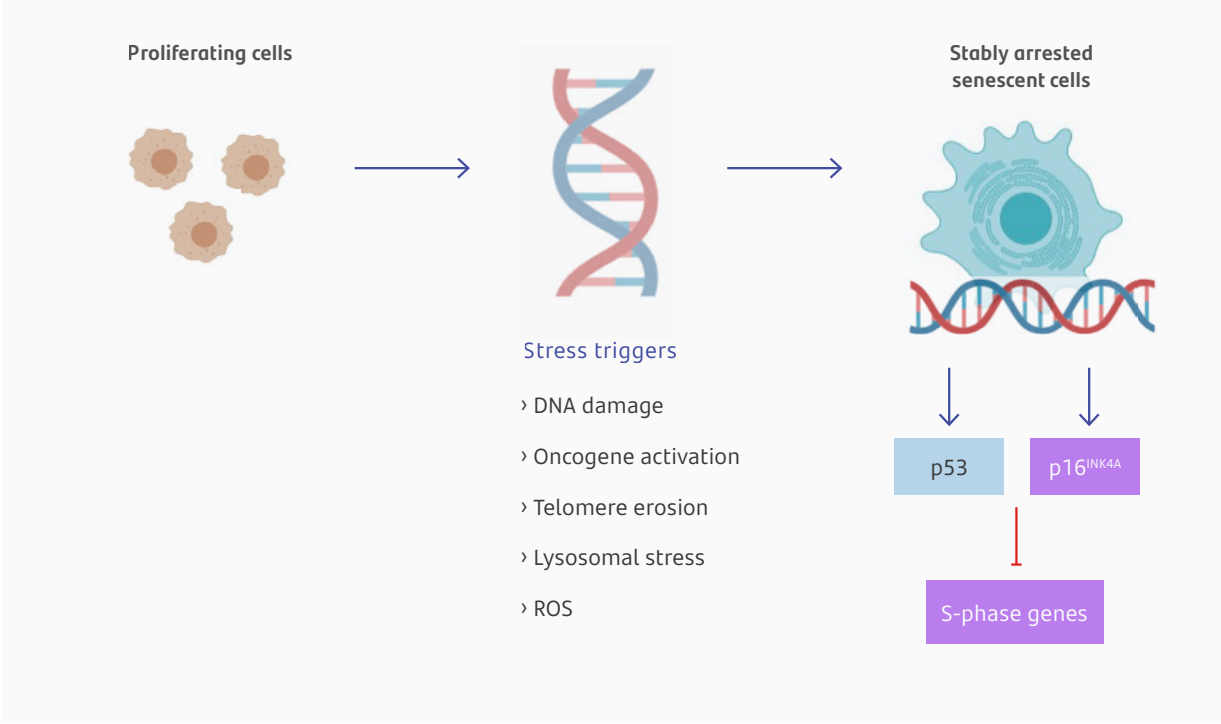
3.1. Senescence as a cellular process

Experiments performed by Hayflick and Moorhead in 1961, led to the initial realization that human cells could only be passaged or split in the laboratory a limited number of times[7]. After that, the cells started to no longer be able to divide and expand and presented marked changes in cell morphology: they became large and flat. This phenomenon was termed replicative exhaustion. Posterior work several decades later by Carol Greider showed that this effect was mediated through the shortening of telomeres in human cell lines; a process known as "replicative senescence"[8]. Additional work by Manuel Serrano, David Beach and Scott Lowe showed that a similar phenomenon could be triggered when cells acquired oncogenic mutations; it was the discovery of "oncogene induced senescence"[9]. Whether senescence was only an in vitro artifact or actually had any physiological relevance was a matter of debate for several decades, however, the identification of senescent cells *in vivo*[10] in 2005 strongly suggested the latter.

Nowadays, it is generally accepted that cellular senescence is a stress response process triggered as a result of DNA damage by which cells with normal proliferative capacity stop dividing and enter into a state of stable cell cycle arrest[11] **(Figure 1)**. At the molecular level, the induction of cellular senescence involves the activation of tumor suppressor genes (such as RB and p53) leading to the silencing of genes linked to proliferation[12-14] **(Figure 1).**

Figure 1: Cellular senescence is a stress response program.

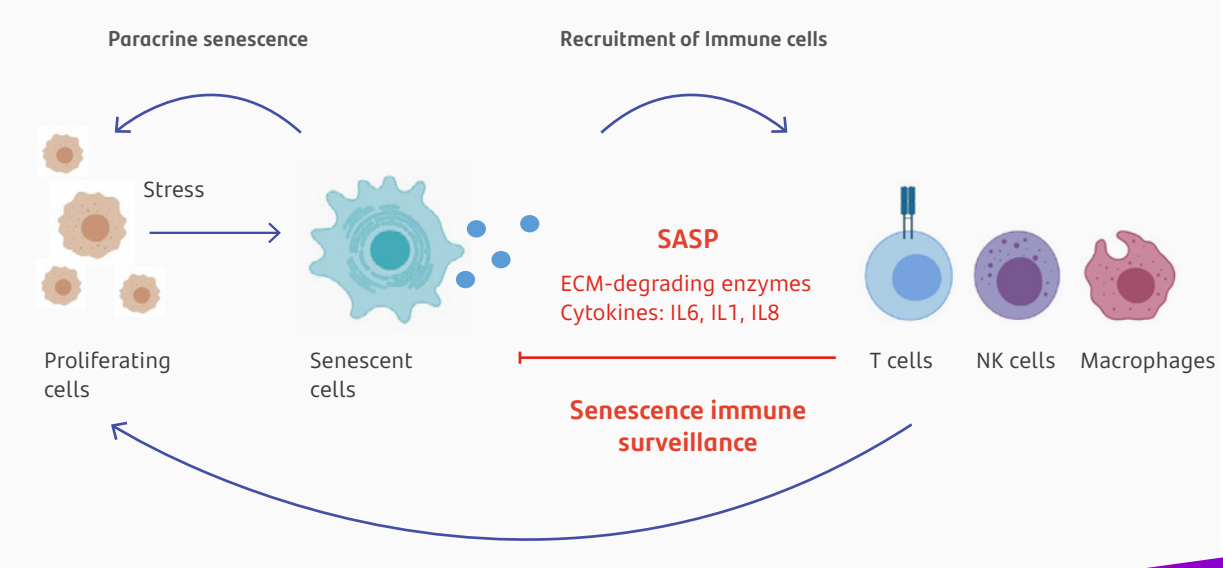
In response to stress, usually in the form of DNA damage because of the activation of oncogenes, telomere erosion or lysosomal stress; proliferating cells enter stable arrest. This process can be triggered through different molecular mechanisms. One possibility is the activation of p53 through the DNA damage response program (DDR)[15]. Other option is the activation of p16INK4A, which controls the activity of cell cycle kinases[11]. Ultimately, both processes can halt cell cycle progression by preventing the transcription of genes required for cell cycle progression[11]. Remarkably, p16INK4a is accepted as a rather specific marker of senescent cells, and has both been used for generating mouse models where senescent cells can be detected or depleted[6,16,17].



In addition, senescent cells are highly proinflammatory and secrete the **"Senescence Associated Secretory Phenotype" (SASP)**[18]. The SASP is composed of proinflammatory cytokines and extracellular matrix remodeling enzymes. It contributes to induce senescence in neighbor cells (paracrine senescence) and recruits immune cells which in turn target the senescent cell restoring tissue homeostasis[19] (the state of balance among all the body systems needed for the body to function correctly), a process known as senescence immunesurveillance[14,20-22]. Both innate and adaptive immunity participate in this process **(Figure 2).**

Figure 2: The Senescence Associated Secretory Phenotype is a key component of senescence biology.

The proinflammatory and extracellular remodeling factors secreted by senescent cells serve two functions: on one hand, they induce senescence in neighbor cells and on the other it recruits immune cells which in turn target the senescent cells. Both arms of the immune system, the innate and the adaptive, are implicated in this process. T cells are part of the adaptive immune system and are implicated in the clearance of senescent cells in premalignant oncogene cells[21], senescent tumors[23] and aging[24]. Natural killer cells (NK cells) are cells capable of recognizing "stressed" cells, such as virus-infected and transformed cells[25]. They are crucial for the elimination of senescent cells in the context of cancer[22,26], liber fibrosis[27] as well as ageing[24]. Beyond NK cells, other cells of the innate immune system such as macrophages have also been shown to play key role in senescence immunosurveillance. Macrophages are important for the elimination of senescent tumor cells[28] as well as senescent cells in the postpartum uterus[29].

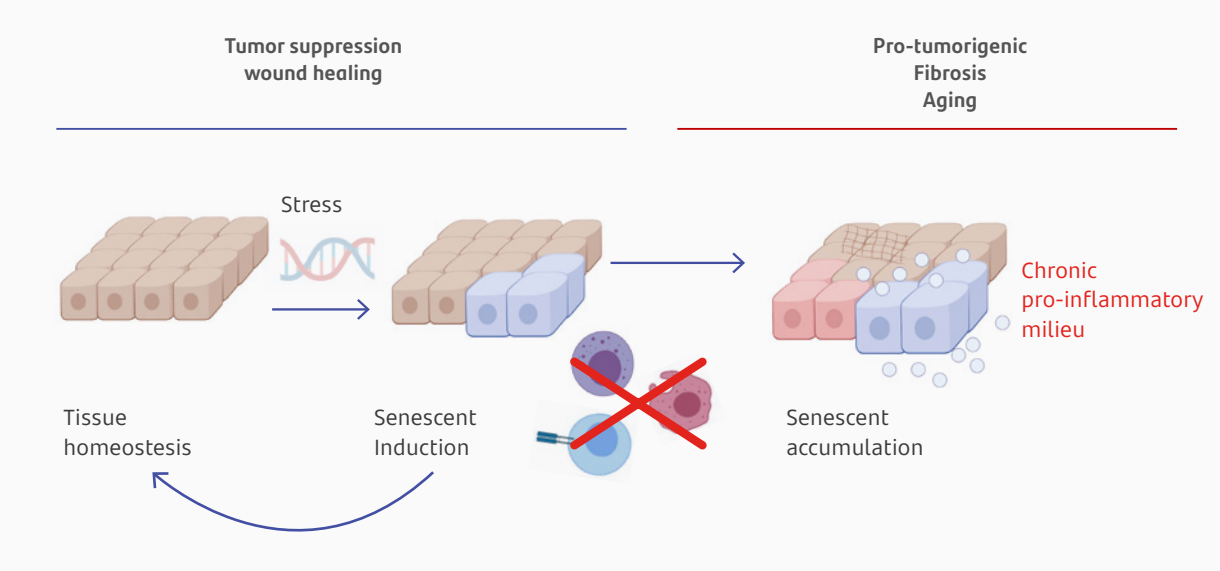


3.2. Beneficial roles of cellular senescence

To comprehend senescence biology, it is crucial to understand it from an evolutionary perspective. It is currently thought that cellular senescence evolved as a tumor suppressor mechanism[30]. Thus, damaged cells bearing oncogenic mutations but not yet fully tumorigenic would trigger the senescence program, secrete inflammatory factors and be eliminated from the tissues by the immune system thereby preventing the possibility of tumor development[30] **(Figure 3)**. Indeed, blocking immune surveillance of senescent premalignant cells significantly increases tumor incidence in animals bearing premalignant lessions[21].

Figure 3: The two sides of cellular senescence.

In physiological conditions, whenever there is damage in the tissue, some cells become senescent and recruit immune cells. In turn, the immune system eliminates these senescent cells and restores tissue homeostasis. This is indeed what happens in the context of tumor suppression or wound healing. However, for mechanisms that remain unclear, in certain settings such as aging the immune system is not able to efficiently eliminate senescent cells at the required rate with the consequence that these cells accumulate in the tissues. This accumulation generates a highly pro-inflammatory milieu that has been shown to contribute to tumor development, fibrosis, accelerated aging and chronic age-related pathologies.



Beyond tumor suppression, cellular senescence has been shown to play key roles in tissue remodeling events such as wound healing[16] and embryonic development[31,32] **(Figure 3)**. Hence, in response to wounds, factors secreted by senescent cells accelerate wound closure[16]. Cellular senescence has been also shown to be important for the correct development of mouse embryos[31,32].

Given the beneficial roles of cellular senescence in younger individuals (embryonic development, tumor suppression, proficient wound healing), and the fact that in nature biological old age is rarely attained[i], there is a strong evolutionary pressure to select for cellular senescence[30]. With age, however, there is both an accumulation of DNA damage, increasing the burden of senescent cells in the organism[33,34], as well as a decrease in the functionality of the immune system itself[24,35,36]. Overall, this leads to the aberrant and deleterious accumulation of senescent cells. **Thus, the process of senescence, which is beneficial in the youth, contributes/drives organismal decay later in life (Figure 3)**.

3.3. Pathological implications of cellular senescence: cancer and age-related pathologies

While the exact molecular mechanisms whereby senescent cells can evade elimination by the immune system remains the subject of intense research; it is known that their aberrant accumulation generates a chronic pro-inflammatory microenvironment that is highly deleterious. Indeed, previous genetic studies have shown that the elimination of certain senescent cells has been shown to improve both healthspan and lifespan in genetic mouse models of accelerated and natural aging[5,6].

Similarly, the presence of senescent cells has been documented in mouse and human samples of chronic age related pathologies such as atherosclerosis, lung and liver fibrosis, diabetes, and Alzheimer's disease, among others[18,37-39]; and their elimination in genetic mouse models has been shown to significantly attenuate disease severity[18,37-39].

Overall, these data strongly support the notion that the accumulation of senescent cells is a key driver in the pathology of aging and chronic age-related diseases and **highlights the need and potential of developing pharmacological and therapeutic strategies** to eliminate or even prevent the accumulation of senescent cells.

i. In nature animals do not typically live up to the potential of their lifespan.

In focus

Identification of Senescent Cells

Importance of biomarkers of senescence

The first step in developing strategies to target senescent cells is to characterize them. Reliable markers of senescent cells would allow to not only detect but also isolate them from tissues in different contexts and study them. In addition, biomarkers are key to 1) identify which patients have high burden of senescent cells and could benefit from therapies and 2) assess non-invasively the effectiveness of the therapy over time.

Current biomarkers of senescence

To date there is no single marker that on itself proves a cell is senescent[11]. Rather, senescence is currently defined by the acquisition of multiple characteristics **(Table 1)**:

Table 1: Markers of senescent cells.

In vitro refers to experiments performed in cell culture in the laboratory. Ex vivo refers to experiments performed with tissues and material isolated from humans and animals. In vivo refers to assays that can be performed in a living organism.

Marker	Description	Technical details	Detection
Morphology	Increased volume and granularity, flat shape		*In vitro:* cell lines
Lysosomal activity	Increased expression of the enzyme ß-galactosidase and detection of its activity at suboptimal pH conditions.		*In vitro:* cell lines *Ex vivo:* tissues
Cell cycle arrest	Lack of cellular division and expression of tumor suppressors	Negative for Ki-67, EdU. $p16^{INK4A}$, Rb, p53, p21.	*In vitro:* cell lines *Ex vivo:* tissues

Marker	Description	Technical details	Detection
DNA damage	Expression of DNA damage response proteins or short and/or dysfunctional telomeres.	SAHF: Senescence Associated Heterochromatic Foci. Expression of DNA damage response proteins: ATM, ATR, 53BP1, yH2AX.	*In vitro:* cell lines *Ex vivo:* tissues
SASP (Senescence Associated Secretory Phenotype)	Proinflammatory cytokines.	IL-1α, IL-6, IL-8, TGFß, MMP1, MMP2, MMP3, MMP10.	*In vitro:* cell lines *In vivo:* organism
Surface markers	Area of ongoing research.	uPAR, DPP4, PD-L2.	*In vitro:* cell lines *Ex vivo:* tissues *In vivo:* organism[ii]

Two of the first described features of senescent cells refer to changes in morphology and the activity of their lysosome, a cellular organelle. Thus, *in vitro* one of the most striking characteristics of senescent cells is their acquisition of a flat and extended shape and increase in their cellular volume and granularity as compared to proliferating counterparts[11]. Another classic and widely used marker of senescent cells both in vitro as well as ex vivo in tissues and cells is the activity of acidic-ß-galactosidase, a lysosomal enzyme that in physiological conditions has an optimal activity at a pH of 4.0-4.5. Senescent cells highly upregulate the expression of this enzyme, to the point that it is possible to detect its activity at suboptimal pH of 5.5 or 6[40].

Another characteristic of senescent cells is their lack of proliferation. Cell cycle arrest can be measured with proliferation assays and is characterized by the high expression of certain tumor suppressors **(see Figure 1)**. In addition, senescent cells bear marks of the DNA damage that triggered the program on the first place, being positive for certain markers[41] and presenting shortened and/or dysfunctional telomeres[8,42].

Finally, the way senescent cells interact with their microenvironment provides additional cues to identify them. On one hand, they secrete the SASP composed of proinflammatory factors[18]. In addition, in recent years there has been growing interest in identifying surface molecules preferentially upregulated by senescent cells (such as uPAR[43], DPP4[44] or PD-L2[45]).

ii. Potentially.

Challenges and future directions

A major limitation regarding the identification of senescent cells relates to the high heterogeneity of the process[46]. Thus, which markers are expressed depends on the cell type and the trigger of senescence[46]. Therefore, there is no one single marker that is consistently upregulated on senescent cells in all cell types. In addition, several of these markers can be expressed by non-senescent cells. Therefore, there is an urgent need to identify more robust and specific markers of senescence. Unlike previous approaches which were too focused on trying to find universal markers of senescent cells, a more limited approach, centered on *identifying the markers expressed by senescent cells on specific tissues in certain pathologies* (e.g.: senescent pancreatic ß cells in diabetes type 2) is more likely to yield highly specific markers for a given setting.

In addition, most of the aforementioned markers allow the identification of senescent cells *in vitro* or *ex vivo*, but few allow to *monitor senescent burden in vivo in a living organism over time*. Potential strategies that are currently starting to be developed in this regard concern imaging technologies and blood biomarkers. Regarding imaging, the physical properties of senescent cells could theoretically allow for their identification by magnetic resonance imaging (MRI). Alternatively, by harnessing surface molecules upregulated on senescent cells, it could be possible to image them through immune-positron emission tomography (ImmunoPET). However, imaging technologies are costly and time-consuming techniques. Blood based biomarkers on the other hand, lack spatial information but could be easily implemented in the clinic by a simple blood draw. Again, while some markers, like soluble uPAR[43] have been proposed as potential plasma biomarkers, more research in future years is necessary to identify additional soluble molecules produced by senescent cells. As with markers, a narrower approach focused on specific settings is more likely to yield specific and clinically applicable blood biomarkers.

04 Strategies to Target Senescent Cells

Strategies to target senescent cells in aging and disease have been collectively termed as *senotherapeutics*. There are currently two kinds of senotherapeutics: senomorphics and senolytics.

4.1. Senomorphics

Concept

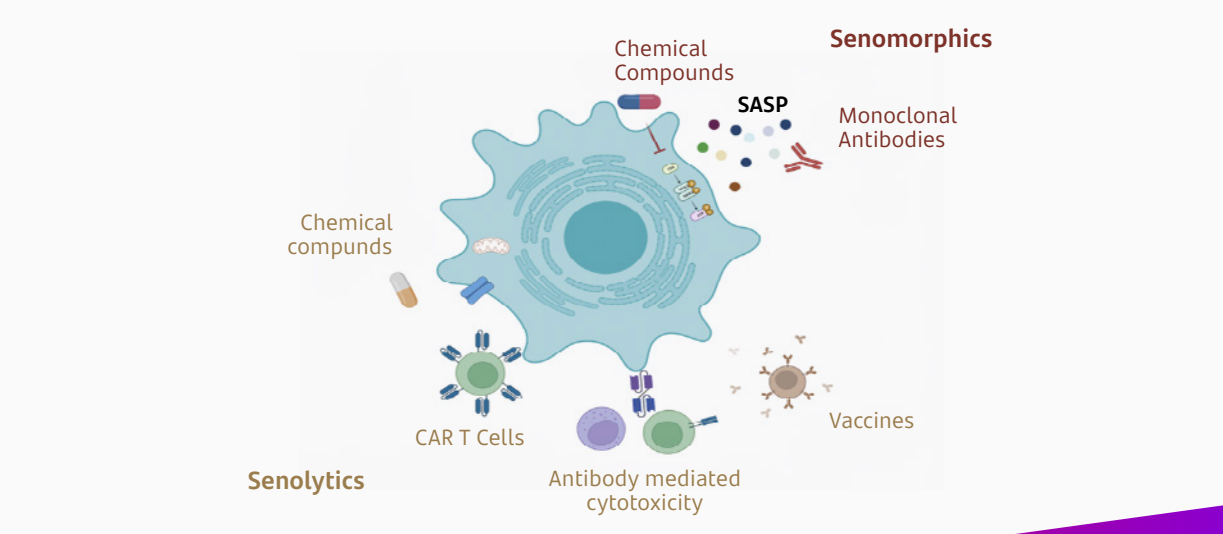
Senomorphics are therapeutics that suppress the secretory phenotype of senescent cells without eliminating the senescent cells themselves. The rationale for their use stems from the notion that silencing their proinflammatory effect on the tissues would be sufficient to obtain therapeutic benefit[47,48].

Current approaches

This class of senotherapeutics can be divided into two major groups **(Figure 4)**. On one hand, chemical compounds that target molecular pathways, transcription factors and epigenetic modulators related to SASP expression[47,48]. On the other, monoclonal antibodies that block specific components of the SASP[47,48].

Figure 4: Overview of senomorphic and senolytic strategies.

Senomorphics aim to block the production or the consequences of the proinflammatory secretory phenotype (SASP) of senescent cells. They can either be chemical compounds that inhibit different molecular pathways inside the cells necessary to produce the SASP or they can be monoclonal antibodies that bind to and block the effect of the factors themselves. Senolytics kill senescent cells. They can be chemical drugs that target vulnerabilities of senescent cells, or immune-based approaches.



Historically, the senomorphic activity of the first chemical compounds was discovered by serendipity. Among these, we find the well-known rapamycin. Originally discovered in soil samples from the Easter Island in 1972, treatment with rapamycin has been shown to mimic calorie restriction and extend healthspan and lifespan in mice[49]. Other compounds in this initial class include corticosteroids, metformin (initially an FDA approved drug for the treatment of type 2 diabetes), resveratrol (an antioxidant) and aspirin. Posterior work focused on the preferential use of small molecules, often, repurposed from anti-cancer therapy. Examples include: ruxotinilib (a JAK1/2 inhibitor initially approved by the FDA for the treatment of myelofibrosis) or JQ1 (an inhibitor of BRD4, an epigenetic remodeler).

Recent efforts have explored the senomorphic potential of using monoclonal antibodies to block either proinflammatory cytokines of the SASP or their receptors[50]. Examples include siltuximab and tocilizumab, or canakinumab and anakinra.

Limitations and future directions

The major weakness of senomorphic approaches is that while they mitigate the effects of senescent cells, they do not tackle the source itself. In addition, by eliminating the proinflammatory part of the SASP they preclude any potential elimination of senescent cells by the immune system. Therefore, they are chronic approaches that require continuous administration over time.

Furthermore, overall, this class of senotherapeutics tends to be unspecific molecules with not entirely known mechanisms of action, likely binding to multiple molecular targets, which limits their potency and increases the risk of side effects. The exception to this would be monoclonal antibodies targeting specific cytokines or their receptors. However, proinflammatory cytokines are also secreted by non-senescent cells (e.g.: most immune cells) and whether the beneficial effects observed can be attributed to blocking the cytokines produced by senescent cells or by simply blocking general inflammation is not known. Moreover, to date, there is not enough research studying which specific SASP factors are key in mediating the pathology associated with senescent cells into each disease in which they had been shown to be implicated.

Altogether, even though senomorphics were the first compounds employed for therapeutically targeting the effect of senescent cells, their limitations (lack of specificity, side effects, chronic administration) made it necessary to develop alternative approaches.

4.2. Senolytics

Concept

Senolytics are therapeutics that directly induce the death of senescent cells[47,48].

Current approaches

To date there are two major classes of senolytics: small molecules that target dependencies (or specific vulnerabilities) on senescent cells to induce cellular death (apoptosis) or synthetic immune-based approaches that lead to the elimination of senescent cells by the immune system (**Figure 4** above).

The first approach relies on the concept, initially developed in cancer therapeutics, of identifying vulnerabilities in senescent cells. For example, being on the verge between apoptosis and senescence, senescent cells tend to upregulate molecules that prevent their death (so called antiapoptic) and depend on their expression for survival. Inhibitors of these molecules have been shown to preferentially kill senescent cells[51]. A challenge of this approach relates to the heterogeneity of senescent cells abovementioned, highlighting the importance of tailoring senolytic approaches to specific contexts. Other examples within this class include cardiac glycosides, which destabilize the membrane potential of these cells and increase intracellular acidification[52]. However, within this class of senolytics we also find molecules, surprisingly widely used in the field, whose mechanism of action and rationale for eliminating senescent cells remains unknown. The combination of dasatinib (a multikinase inhibitor) and quercetin (a flavonoid) to target cells that are stably arrested is a good example of this[53]. Despite promise in preclinical studies, clinical trials using chemical senolytics of this class have had only mixed results to date[54].

Recent work has started to explore the notion of harnessing the immune system to eliminate senescent cells. This approach is inspired by the effective elimination of senescent cells by the immune system in younger physiological conditions. Thus, the identification of surface markers upregulated on senescent cells opens the door to strategies that redirect the endogenous immune system to target them like antibody-directed cellular cytotoxicity[44] (that recruits NK cells) or Bi-specific T-cell engagers (BiTEs) (that recruits T cells[55]). When restoration of endogenous immunosurveillance is not possible, however, a powerful alternative could be the use of synthetic immunity, in particular cellular therapy, such as the use of Chimeric Antigen Receptors (CARs), synthetic receptors that target immune cells to specific cell surface antigens redirecting their effector potential[56,57].CAR T cells targeting the B cell surface antigen CD19 which have shown remarkable efficacy in patients with B cell malignancies (such as non-Hodgkin lymphomas or chronic lymphocytic leukemia)[58]. Other cell surface antigens have shown promise as targets for CAR T therapy in diseases beyond cancer such as autoimmune settings[59,60] or chronic infections[61]. Recently, the first CAR T cells targeting senescent cells were developed[43,62].

Limitations and future directions

A general challenge in developing senolytic approaches relies on the heterogeneity of senescent cells, which manifests as having different genetic dependencies or surface protein expression in a context-dependent manner[46]. This heterogeneity limits potency of both chemical and immune-based approaches and future research should focus on identifying and tailoring senolytic strategies to specific diseases.

In addition, specificity of the effects, this is, killing only senescent cells but sparing healthy cells in the tissue, is a major challenge of chemical-based approaches. However, recent promising strategies have tried to redirect the action of the compounds towards senescent cells by employing pro-drugs and nanoparticles. For example, since senescent cells have increased lysosomal SA-ß-Gal activity, one strategy could be to couple the compounds to a cleavable galactose moiety[63] or to encapsulate them into galacto-oligosaccharides nanoparticles[64] so that the drugs would be preferentially uptaken and activated by senescent cells. Another approach that could be used to lower drug dose and limit toxicity is the use of proteolysis targeting chimeras (PROTACs). Unlike traditional drugs, PROTACS are highly selective for specific targets significantly increasing the efficacy[65].

Regarding immune based approaches, the major limitation concerns the identification of surface molecules specifically upregulated on senescent cells. One possibility could be the incorporation of CARs or antibodies with dual specificities using and/or logic gate approaches[57]. Indeed, a major advantage of cellular therapy and CARs is the versatility of the system and future work should focus on identifying the optimal CAR design in terms of safety and efficacy. In addition, to limit potential toxicities the incorporation of safety switches could minimize them and provide and additional layer of control.

Moreover, research into the surface molecules that senescent cells upregulate will also likely help to shed light into the mechanisms of senescence immune surveillance and their evasion by immune cells, which could contribute to new therapeutic avenues.

Case in point

Novel therapeutic avenues: is prevention possible?

So far research in senotherapeutics has focused on treatment strategies, but could it be possible to prevent the accumulation of senescent cells in the first place? Recent work has explored the idea of vaccinating against senescent cells by harnessing antigens present exclusively or preferentially on these cells[66]. While preliminary, preclinical research does support the feasibility of this approach[66] as long as antigens could also be found on human senescent cells.

In addition, unlike any drug-based approach, cellular therapy has the potential to develop memory CAR T cells and persist for decades after single administration[67]. Thus, it is possible that senolytic CAR T cells could also mediate long term and even preventive therapeutic effects for years after only one dose. This could have a significant impact in the quality of life of patients with chronic age-related diseases that nowadays require chronic daily treatment and would provide a cost-effective rationale for the use of these costly therapies to tackle senescent cells.

Case in point

Senolytics in Biotechnology

The senolytic space in biotechnology is novel and companies in this sector are still young. To date, all public companies are based on small-molecule based approaches. The first one to launch clinical trials was Unity Biotechnology. This company is investing in the combination of dasatinib and quercetin (unspecific senomorphic and senolytic) and the compound UBX1325 (an inhibitor of Bcl-xL which is an inhibitor of an antiapoptotic molecule). Clinical results have been mixed to date with one failed phase 2 clinical trial on osteoarthritis (NCT04129944) and several other trials still ongoing. Other companies developing similar approaches are FoxBio, Numeric Biotech, Rubedo Science and Juvenescence.

While most efforts are focused on senolytics, there are some companies investing as well on senomorphics such as: Senisca, Atropos Therapeutics and Dorian Therapeutics.

An underdeveloped area in senolytics biotech to date are immune-based approaches. There are currently a few companies in the works focused specifically on developing CAR T cells against senescent cells, but this is still an open field. Other cellular therapy-based approaches as well as antibody or immune checkpoint strategies could hold promise. As mentioned above, immune-based strategies are interesting because they offer higher specificity which translates into better efficacy and safety and unlike small molecules, they have the potential for long-term benefits. However, they do suffer from significantly higher production costs.

An interesting case given its wider portfolio is Senolytic Therapeutics which are developing inhibitors of an antiapoptotic molecule as senolytics but also have a senomorphic approach and have recently patented an immune-based approached focus on immune checkpoint inhibition.

Finally, beyond a specific focus on senescence, it is worth mentioning two major companies working on aging research from a more general perspective (including senescence) such as Altos Labs (whose focus is regeneration) or Calico. Both of which have considerable capital and have recruited exceptional scientific leaders to support their efforts.

Overall, the senolytic biotech is a new space which warrants further growth in the coming years. As the world population ages, there is increased demand (and rationale) for these therapies that could be used to treat multiple age-related pathologies in the same way that currently several cancers are treated by mutational status instead of tissue of origin.

05

Conclusion: future directions in senescence research and senotherapeutic development

Overall, while progress has been made in the development of therapeutic strategies that target senescent cells; significant investment is needed to optimize the generation of potent and cost-effective approaches.

The major limitation of senomorphics and small molecule senolytics relies on the lack of specificity of most of these compounds which limits their potency and efficacy. On the other hand, they are more affordable and easier to administer to patients. Future work focused on developing highly specific molecules using PROTAC or other chemical approaches would improve their profile, although this would require the identification of robust vulnerabilities of senescent cells across settings. Strategies to reroute their action only to senescent cells through nanoparticles or even antibody-based approaches could also enhance their efficacy and safety. This would bring (or merge) this approach closer to the immune-based strategies, which, on the other hand, are very precise and potent but suffer from larger costs. However, if cellular therapy-based approaches could indeed drive long term effects (for years) after single administration, the cost-effectiveness profile of these products could change.

Finally, beyond current approaches, an understudied area in cellular senescence is its connection with the other hallmarks of aging[4]. Thus, while significant efforts have been dedicated to characterizing the individual contributions of each hallmark to aging, today little is known about the interactions among them. Whether there are hierarchies among the hallmarks, temporal and spatial determinants of these interactions, or how they are modulated in age-related pathologies remains unknown. Understanding these dynamics could also provide new alternatives to target senescent cells in aging.

Annex 1. Current senomorphics and described mechanism of action

Type of senomorphic	Class of compound	Example of compounds	Heat
Chemical compounds		Mechanism of action	Unclear, inhibition of mTOR
		Metformin	Unclear, inhibition of NK-κB, AMPK, STAT3...
		Resveratrol	Unclear, inhibition of SIRT1, NK-κB
		Aspirin	Unclear. Inhibition of COX1/2
	NK-κB inhibitors	Corticosteroids SR12343	Inhibits NK-κB
	JAK/STAT inhibitors	Ruxolitinib	Inhibits JAK1/2
	Statins	Atovarstatin, pravastatin, simvastatin	Inhibits HMG-CoA reductase
	ATM inhibitors	KU-55933, KU-60019	Inhibits DNA damage response protein ATM
	BET inhibitors	JQ1	Inhibits BRD4
	Natural products	Quercetin	Flavonoid, activates the proteasome
Monoclonal antibodies		Siltuximab Tocilizumab Canakinumab Anakinra	Inhibits IL-6 Inhibits IL-6 receptor Inhibits IL-1ß Inhibits IL-1ß receptor

Annex 2. Current senolytics and described mechanism of action

Type of senomorphic	Class of compound	Example of compounds	Heat
Chemical compounds	BCL-2 inhibitors	ABT-263, ABT737	Disrupts FOXO4-p53 interaction.
	Targeting p53	FOXO4-DRI	Inhibits NK-κB
	HSP90 inhibitors	Ganetespic, Geldanamycin	Inhibits HSP90
	Cardiac glycosides	Proscillaridin A, Ouabain, Digoxin, Bufalin	Inhibits Na+/K+ ATPase
	Multikinase inhibitor	Dasatinib	Preferentially inhibits BCR-ABL
	Natural products	Quercetin Fisetin	Flavonoids. Unclear
Antibody mediated cytotoxicity		Antibodies against DPP4	Antibodies bind to DPP4 positive cells and NK cells are recruited to eliminate them
Vaccines		CD153 vaccine GPNMB vaccine	Antibodies are generated that eliminate CD153 positive cells. Antibodies are generated that eliminate GPNMB positive cells.
CAR T cells		Anti-uPAR CAR T cells	Eliminate uPAR-positive cells.
		Anti p16-MHC CAR T cells	Eliminate p16-MHC positive cells.

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