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Review Article

Cellular senescence and the host immune system in aging and age-related disorders

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ABSTRACT

Cellular senescence is a complex process involving a close-to-irreversible arrest of the cell cycle, the acquisition of the senescence-associated secretory phenotype (SASP), as well as profound changes in the expression of cell surface proteins that determine the recognition of senescent cells by innate and cognate immune effectors including macrophages, NK, NKT and T cells. It is important to note that senescence can occur in a transient fashion to improve the homeostatic response of tissues to stress. Moreover, both the excessive generation and the insufficient elimination of senescent cells may contribute to pathological aging. Attempts are being made to identify the mechanisms through which senescent cell avoid their destruction by immune effectors. Such mechanisms involve the cell surface expression of immunosuppressive molecules including PD-L1 and PD-L2 to ligate PD-1 on T cells, as well as tolerogenic MHC class-I variants. In addition, senescent cells can secrete factors that attract immunosuppressive and pro-inflammatory cells into the microenvironment. Each of these immune evasion mechanism offers a target for therapeutic intervention, e.g., by blocking the interaction between PD-1 and PD-L1 or PD-L2, upregulating immunogenic MHC class-I molecules and eliminating immunosuppressive cell types. In addition, senescent cells differ in their antigenic makeup and immunopeptidome from their normal counterparts, hence offering the opportunity to stimulate immune response against senescence-associated antigens. Ideally, immunological anti-senescence strategies should succeed in selectively eliminating pathogenic senescent cells but spare homeostatic senescence.

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Introduction

The transition from a healthy to a diseased state is driven by perturbations in cellular fitness that – individually or together – determine the age-dependent decay of organismal functions. Among the different “hallmarks of aging”, in the last decade cellular senescence has entered the limelight due to its key involvement in the pathogenesis of major age-related illnesses [1].

Cellular senescence is characterized by a stable proliferative arrest that can be triggered by multiple stimuli, spanning from developmental cues to cellular stresses including – but not limited to – DNA damage, telomere attrition, persistent activation of trophic signals, oncogenic activation, and oxidative stress. These signals intercept multiple effector pathways, most of which converge on the activation of the p53-p21 and p16-RB axes that in turn act as cell cycle regulators, preventing the proliferation of damaged or stressed cells, usually in an irreversible fashion [2]. The senescent phenotype is characterized by peculiar morphological, epigenetic, and metabolic changes that favor the aforementioned cell cycle arrest, but also entail resistance to apoptotic cell death and a prominent proinflammatory secretome, referred to as senescence-associated secretory phenotype (SASP) [3]. SASP is triggered by a multipronged signaling network comprised of DNA damage response (DDR), p38/MAPK, inflammasome and mTORC1, ultimately converging on NF- κ B and C/EBP β -dependent transcriptional programs. The SASP exerts potent effects on neighboring cells and surrounding tissues. As an example, the SASP was found to exacerbate inflammation, promote angiogenesis, ignite the fibrogenic cascade and favor cell proliferation. The cell type, context-dependent and dynamic attributes of the SASP are critical determinants of the beneficial or deleterious effects of senescent cells in the organism [4].

There is a sizable body of literature reporting that senescent cells progressively accumulate in the tissues of experimental model organisms (such as mouse and turquoise killifish) and humans during chronological aging [5–7]. In support of the causal nexus between the excessive burden of senescent cells and aging, the genetic ablation of p16/Ink4a⁺ senescent cells was shown to extend healthspan and lifespan of progeroid and naturally aged mice [8,9]. Conversely, senescent cells are sufficient to promote the manifestation of systemic or tissue-specific dysfunctions linked to aging when transplanted into otherwise healthy/young animals [10]. These results have sparked considerable interest towards the discovery or repurposing of drugs for use as ‘senotherapeutics’ in preclinical disease models and in the clinic. The arsenal of senotherapeutics [11] available for (pre)clinical testing currently consists of ‘senopreventive’ drugs, senomorphics (i.e., agents that modify specific aspects of the senescent phenotype without eliciting direct lethal effects to senescent cells) and senolytics, defined as molecules that trigger the selective apoptosis of senescent cells [12].

The molecular and phenotypic changes that underlie the conversion of a normal cell into a senescent cell endow the latter with the ability to crosstalk with host leukocytes. Consistent with this notion, adaptive and innate immune cells directly control the burden of senescent cells in tissues, being

directly implicated in both the ignition and the extinction of the process [13]. In this review, we provide an overview on the molecular basis regulating the dialogue between immune cells and senescent cells, and we explore the pathophysiological consequences of this interaction in aging-associated phenotypes and antisenesescence therapies.

Senescence in physiology and pathology: one size does not fit all

The conjecture that the generic term ‘senescence’ fails to fully recapitulate the transcriptional and functional heterogeneity of the senescent state has recently gained traction [14,15]. While individual phenotypic features of senescent cells (e.g., heightened p16 or p21 expression, enhanced senescence associated beta galactosidase activity) – or a combination thereof – are routinely employed to isolate and study senescent cells, these markers are often unable to distinguish ‘adaptive’ senescence from its ‘maladaptive’ counterpart. In contrast to the well rooted knowledge that pathogenic senescent cells are abundant in aged or diseased tissues (see below), cells scoring positive for common markers of senescence are physiologically (yet often transiently) present in the tissues of young/healthy individuals, where they play important roles in processes such as wound healing [16], regeneration [17], tissue reprogramming [18] and limitation of fibrotic scarring [19]. As an example, p16/Ink4a⁺ cells are detected in the basement membrane of the lung where they contribute to tissue regeneration and repair after damage [20]. Even in the context of natural aging, elimination of specific subtypes of senescent cell may elicit deleterious effects, as testified by the fact that elimination of p16/Ink4a⁺ liver sinusoidal endothelial cells disrupts vascular structure and leads to perivascular fibrosis [21].

It has been recently proposed that the concept of ‘beneficial’ senescence may be interchangeable with that of transient senescence, in thus far that ‘adaptive’ senescent cells are rapidly cleared by host leukocytes cells shortly after their formation, paralleling the reestablishment of tissue homeostasis [15]. In line with this tenet, preneoplastic p21⁺ cells detected in the liver following KRAS^{G12V} activation are placed under immunosurveillance and progressively cleared by host immune cells shortly after induction [22]. In a similar vein, p21⁺ cells generated during normal embryonic development are only transiently detectable, as they undergo macrophage-assisted elimination [23].

In stark contrast, pathogenic senescent cells persist in aged or damaged tissue with longer latency than their beneficial counterparts, at least in part by suppressing the cytotoxic action of immune cells in charge of their surveillance. Importantly, these persister variants display the ability to propagate the senescent phenotype (and hence induce ‘secondary or paracrine senescence’) to other cells laying in the same tissue or located at distal sites, largely via their bioactive secretome [4]. The accumulation of senescent cells during aging has been recently quantitated longitudinally across multiple tissues from wild type (WT) naturally aged mice and *Erc1*^{-/-} progeroid mice, which lack the DNA repair enzyme ERCC1 [6]. In both models, markers of senescence were found

elevated in peripheral T cells and in most organs except the heart and skeletal muscles, aorta being the tissue with the most pronounced accrual of senescent cells. The load of senescent cells significantly ramped up after mice reached adulthood and continued to increase over time. Interestingly, a sex-dependent pattern of senescent cells accumulation was found, with female mice presenting a greater increase in the rate of senescent cells formation towards geriatric age [6].

The generation of transgenic mouse models (e.g., INK-ATTAC, p16-3MR) to induce the selective apoptosis of p16^{high} cells shed light on the preclinical value of reducing the burden of senescent cells in contexts of premature/natural aging [8,16]. Pioneering work by Baker and colleagues first reported that the removal of senescent cells extended the lifespan of progeric BubR1 hypomorphic mice [8]. The same group also described that the systemic ablation of senescent cells was sufficient to prolong the lifespan of naturally aged INK-ATTAC mice while attenuating the occurrence of life-threatening diseases (including cancer and cardiovascular diseases) across adulthood [9]. In line with these data, the genetic ablation of p16⁺ senescent cells in the INK-ATTAC model mitigated age-dependent bone-loss [24] and promoted rejuvenation (as mirrored by improved cognitive function and reduced markers of chronic inflammation) of the aged brain [25]. In analogy with this finding, the intermittent clearance of p21^{high} cells improved parameters of systemic health in 23-month-old animals [26].

Notably, phenotypic improvements associated with the removal of senescent cells were also observed in murine models of tauopathies [27], atherosclerosis [28], metabolic diseases [29,30], as well as in the context of iatrogenic senescence induced by systemic doxorubicin treatment [31].

Importantly, most of the beneficial effects reported in transgenic mouse models have been validated by pharmacological interventions. As an example, senolysis induced by the Bcl-XL inhibitor navitoclax enhanced tissue (e.g., bone marrow, muscle) function by eliminating aged stem cells [32] and reestablished metabolic homeostasis in murine models of metabolic syndromes [30]. Similarly, navitoclax administration restored kidney regenerative capacity of aged mice or animals exposed to sub-lethal irradiation following ischemia/reperfusion injury [33]. Peptides designed to disrupt FOXO4-p53 interaction and induce p53-dependent apoptosis of senescent cells alleviated signs of systemic toxicity induced by doxorubicin and extended the lifespan of progeroid and naturally aged mice [34]. The combination of dasatinib and quercetin was associated with improved disease outcome in multiple preclinical models including metabolic syndromes [35], intestinal dysbiosis and inflammation [36], spinal cord injury [37], Alzheimer disease [38], osteoarthritis [39], sarcopenia [40], severe acute respiratory syndrome coronavirus 2 [41], as well as pulmonary [42] and renal fibrosis [43]. Health-promoting effects associated with the clearance of senescent cells *in vivo* were also described for glutaminolysis inhibitors [44], cardiac glycosides [45] and fisetin [41,46]. Consistently, chronic senolytic treatment with D + Q or fisetin led to a moderate but significant extension of lifespan and healthspan in mouse models of natural aging [10,46].

Noteworthy, there is (limited) preliminary evidence that senolytic administration may reduce senescent cells burden

in patients [47,48]. In this regard, results from randomized clinical trials will be instrumental to substantiate the pre-clinical findings. In the light of the spurious mode of action of these agents, it remains nonetheless debatable that positive effects of these agents on parameters of organismal health would exclusively depend upon the reduction of the burden of senescent cells in tissues. Furthermore, it will be critical to compare side-by-side the pro-longevity effects of senolytics with those evoked by senomorphic-based approaches - which encompass well known geroprotectors such as rapamycin [49], physical exercise [50], metformin [51,52], and caloric restriction [53] - in order to design optimal antisenescence therapies for disease prevention and treatment. Interestingly, both senolytic and senomorphic compounds can be found in edible sources, suggesting that the senescent phenotype can be modulated *in vivo* via the diet [54]. Nonetheless, it remains to be demonstrated that these molecules can reach therapeutic concentrations via mere dietary intake.

Interplay between senescent cells and host immunity

Cell-intrinsic events linked to the ignition of senescence influence organismal functions in a non-cell autonomous manner - via the senescent secretome or the release of extracellular vesicles [55]. As an example, sensing of cytosolic chromatin fragments or free mitochondrial DNA by the cyclic GMP - AMP synthase (cGAS) following DNA damage or oxidative stress promotes the STING-dependent production of core SASP protein [56]. Likewise, defective autophagy, excessive mTORC1 activity and inflammasome hyperactivation support the production of IL1A, which regulates SASP in an autocrine and paracrine manner [57,58]. As yet another example, “damaged” or senescent cells use exosome secretion to eliminate harmful cytosolic DNA, which acts as a damage associated molecular pattern (DAMP) on target cells [59].

The release of SASP mediators sets the ground for the termination of the senescence-driven tissular response. This is achieved by the chemoattraction of immune cells in charge of senescent cells elimination. With a considerable degree of context-dependency, both innate and adaptive immune cells may place senescent cells under surveillance (summarized in Fig. 1).

As first reported in settings of malignant transformation, innate immune cells are primarily implicated in the elimination of senescent cells. In a murine model of hepatocellular carcinoma, inflammatory cytokines (i.e., IL15, CSF1, MCP1, CXCL1) released by senescent tumor cells following p53 reactivation promoted tumor eradication by natural killer (NK) cells, neutrophils, and macrophages [60]. Likewise, TH1 cytokines released by CD4⁺ T lymphocytes favor macrophage-dependent elimination of pre-neoplastic tumor lesions in the liver [61]. Furthermore, pharmacological, or genetic interventions that trigger senescence in a large fraction of malignant cells limit tumor outgrowth in a NK and T-cell dependent manner [62,63].

At the present stage of the literature, there is compelling evidence in support of the role of NK cells as ‘immune senolytics’, even in the context of non-oncological illnesses. In a

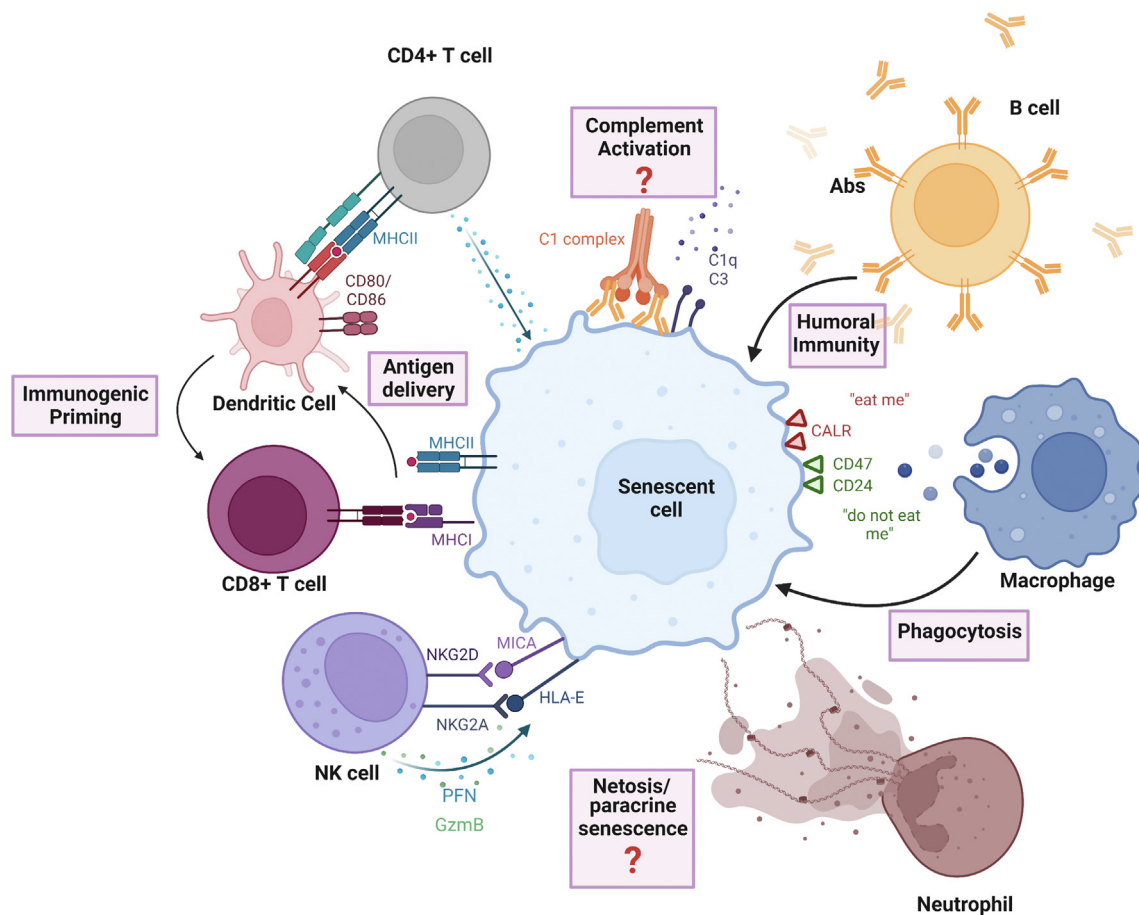


Fig. 1 Overview of the senescent–to-immune cells crosstalk. The profound perturbations in the biology of senescent cells render them able to communicate with both the innate and the adaptive branches of the host immune system. In turn, different population of host leukocytes participate in the surveillance of senescent cells. Some of the immunological effects elicited by senescent cells -including the overproduction of complement factors or their dialogue with neutrophils remain to be characterized.

Abbreviations: Abs: Antibodies; MHC: Major Histocompatibility Complex; NK: Natural Killer; NKG2D: natural killer group 2D; NKG2A: natural killer group 2A; MICA: MHC class I polypeptide–related sequence; PFN: Perforin; GzmB: Granzyme B; HLA-E: Major Histocompatibility Complex; Class I, E; CALR: Calreticulin; CD47: Cluster of Differentiation 47; CD24: Cluster of Differentiation 24.

seminal work, Krizhanovsky and colleagues demonstrated that induction of senescence in activated stellate cells – followed by rapid NK-mediated killing – was instrumental to limit the fibrogenic cascade in the liver [19]. Mechanistically, the expression of activating NKG2D ligands (i.e., MICA, ULBP2) on the surface of senescent cells unleashes the cytotoxic activity of NK cells [64]. NK cells eliminate senescent cells via granule exocytosis [65], as demonstrated by the fact that perforin-deficient mice exhibit elevated accumulation of senescent cells in tissues coupled to chronic inflammation and shortened lifespan [66].

More recently, invariant natural killer T (NKT) cells were also assigned a role in the clearance of pathogenic senescent cells from tissues. Thus, iNKT stimulation by glycolipid antigen α -galactosyl ceramide (α GalCer) was sufficient to reduce the number of senescent cells in the fibrotic (bleomycin-treated) lung and in white adipose tissue (WAT) from high-fat diet fed mice [67].

Macrophages also participate in the elimination of senescent cells, although the ‘eat me’ versus ‘do not eat me’ signals involved in the process remain elusive. Macrophage-dependent senescent cell clearance was consistently reported in pathophysiological instances of limb regeneration in salamanders [68], embryogenesis [23], postpartum uterus remodeling [69] and cancer [60]. Under circumstances of chronic liver damage, a p53-dependent senescence program in stellate cells induces the polarization of macrophages towards an M1 proinflammatory phenotype, endowed with phagocytic ability towards senescent cells [70]. Human senescent fibroblasts transplanted into the peritoneal cavity of severe combined immunodeficiency disease (SCID) mice (which lack adaptive immunity) were rapidly eliminated by phagocytes. Conversely, when human fibroblasts are protected from clearance following encapsulation into alginate beads, a sub-class of macrophages with features of senescence was attracted to the site of injection, suggesting that

senescent-like macrophages may functionally contribute to the aged phenotype elicited by senescent cells [71]. In addition, SASP factors released by senescent cells promote the upregulation of the ecto-enzyme CD38 on the surface of macrophages. In turn, CD38 overexpression by macrophages reduces the systemic availability of NAD^+ and instigates the chronic production of inflammatory cytokines, two events that precipitate the aging phenotype [72].

Recently, a link between the immunosurveillance of senescent cells by neutrophils has emerged. In a murine model of retinopathy, the production of neutrophil extracellular traps (NETs) is instrumental for the clearance of senescent endothelial cells and the consequent vascular remodeling [73]. By contrast, neutrophils recruited to the damaged or aged liver following the release of SASP factors by senescent cells contribute to the paracrine spreading of the senescent phenotype to neighboring cells via oxidative damage-dependent telomere shortening [74]. This result suggests that neutrophils may play a key role in the systemic propagation of the senescence phenotype observed during aging.

In multiple independent datasets (including transcriptomics and proteomics) generated from diverse models of senescence *in vitro*, a strong correlation between the senescent state and the overexpression of core components of the complement system (e.g., C3) has surfaced [75,76]. While the pathophysiological relevance of the excessive production of complement factors by senescent cells *in vivo* remains to be studied, it is tempting to speculate that senescent cells contribute to the overexuberant activation of the complement cascade, which reportedly occurs during aging [77] and in pathologies (e.g., chronic kidney disease) in which senescence has a well-defined pathogenic role.

Both humoral and cellular forms of adaptive immunity have been implicated in the recognition and elimination of senescent cells *in vivo*. Albeit additional evidence is required to validate this finding, immunization of Balb/c mice with senescent lung fibroblasts triggered the production of IgM antibodies against a plasma membrane-bound oxidized form of vimentin expressed on the surface of senescent cells. Moreover, senescent cells may be sensitive to antibody-dependent cell-mediated cytotoxicity following production of natural antibodies against surface antigens [78].

As previously mentioned, cytokines produced by antigen specific CD4^+ T cells in pre-neoplastic tumor lesions are propaedeutic for the macrophage-assisted clearance of senescent cells [61]. Consistently, melanocytes undergoing oncogene induced senescence (OIS) trigger a CD4^+ T cell-dependent response mediated by the upregulation of MHC-II presentation machinery and the concomitant overexpression of MHC-II molecules on their surface [79].

Interestingly, macrophages infiltrating the liver upon CXCL14 production by p21^+ hepatocytes expressing $\text{KRAS}^{\text{G12V}}$ facilitate the CD8^+ T cells dependent killing of these preneoplastic cell variants [22]. In an experimental model of post-traumatic osteoarthritis, a functional correlation between senescence markers and pathologically elevated levels of interleukin-17 (IL17) was established, at least in part tied to the ability of senescent fibroblasts to skew naïve CD4^+ T cells towards a Th17 phenotype in a TGF-beta dependent manner. Interestingly, elevated levels of IL17 directly promoted the

paracrine spreading of senescent cells in the articular joint [39].

The molecular bases of the crosstalk between CD8^+ T cells and senescent cells are being unveiled. It has been recently reported that - regardless of their transformed status - senescent cells mounted a potent CD8^+ T cell-dependent immune response when transplanted into immunocompetent hosts. Based on these results and previous data, it can be speculated that recognition of senescent cells by CD8^+ T lymphocytes depends upon multiple mechanisms including (i) SASP; (ii) enhanced expression of MHC-I and MHC-I presenting machinery; (iii) the emissions of DAMPs at a level sufficient to promote the recruitment and maturation of antigen presenting cells (APCs); (iv) the efficient transfer of antigens to APC; and (v) an altered immunopeptidome, resulting in the presentation of unique antigens not expressed by their normal counterparts and capable of inducing effective memory response [63,76].

Immunological mechanisms of senescent cell accumulation

The physiological triggers of senescence during natural aging remain elusive. Recent findings suggest that persistent levels of trophic signals (e.g., insulin) or episodes of vascular damage may contribute to the increased load of senescent cells *in vivo* [52,80]. As observed in experiments of heterochronic parabiosis, transfer of bloodborne pro-geronic factors from old to young animals is sufficient to increase the number of senescent cells in tissues [81,82]. Concurrently, old mice exposed to the young circulatory milieu showed dwindling levels of senescence markers [82]. Aligned to these results, removal of senescent cells by senolytic treatment prior to blood exchange annihilated the pro-aging phenotype in young mice [82]. It is therefore plausible (as recently theorized [83]) that the elevated age-associated burden of senescent cells would stem from a combination of increased formation (due to time-dependent accumulation of damage) and decreased elimination, the latter being linked to strategies of evasion from immunosurveillance or a decline in the fitness of immune cells responsible for senescent cell removal.

Data inferred from two recently generated models of genetic-induced “immunosenescence” reinforce the hypothesis that a dysfunctional/aged immune system would act as primary driver of systemic senescence. Accelerated aging of the immune compartment through the ablation of ERCC1 in hematopoietic stem cells – which led to increased DNA damage and senescence of leukocytes population including NK cells and follicular T helper cells – was sufficient to cause the systemic elevation of the senescence markers p16 and p21 during aging. This effect may be explained by inefficient immune clearance of senescent cells [84]. Similarly, induction of premature CD4^+ T cell aging features by the specific obliteration of TFAM – a key regulator of mtDNA replication and transcription – promoted multi-organ (liver, heart, and gonadal white adipose tissue) accumulation of senescent cells. This effect is mediated by the excessive production of inflammatory cytokines (and notably $\text{TNF}\alpha$) by dysfunctional T cells [85].

Collectively, these studies insinuate the possibility that “immunosensescence” may temporally precede - and hence play a causal role - in the pathological accrual of senescent cells in non-lymphoid tissues. However, the immunosenescence phenotype [86] is complex in thus far that it involves thymic involution (accompanied by reduced production of naïve T cells and expansion of specific memory T cells clones) and myeloid-over-lymphoid bias in central hematopoietic organs driven by aberrant age-related clonal hematopoiesis. Thus, additional work in more physiological setups is needed to clarify which features of immunosenescence contribute to the multi-organ accumulation of senescent cells. In turn, this would pave the way to approaches that effectively rejuvenate the immune system in a way that systemic aging is halted or reversed. In this regard, it is tempting to speculate that interventions known to improve immune cell fitness – such as caloric restriction, caloric restriction mimicking diet or exercise – would elicit beneficial systemic effects due to enhanced senescent cell immune clearance.

The persistence of senescent cells in aged or damaged tissues involves immunosuppressive strategies orchestrated by these persisters variants to resist immune disruption. Similar to what has been previously reported in the context of neoplastic malignancies, senescent cells may bypass immunosurveillance via the expression of secreted or cell surface-bound molecules (henceforth referred to as senescence associated immune checkpoints, SAICs) that directly inactivate the cytotoxic activity of immune cells in charge of their surveillance. In addition, senescent cells can elicit perturbations in the microenvironment due to the local attraction of diverse immunosuppressive immune subsets, which in turn hamper the cytotoxic function of innate and adaptive immune cells. In support of the latter scenario, phenomena of senescence-related ‘immunosuppressive inflammation’ have been thoroughly described in the different mouse models of cancer, in which senescent neoplastic or stromal cells skewed adaptive immune responses via the recruitment of myeloid derived suppressor cells (MDSC) [87]. It is plausible – yet remains to be proven - that such phenomena may also occur during natural aging or in age-related diseases.

With a high degree of context dependency, a number of SAICs have now been described. Seminal works from the group of Irving Weissman and others shed light on the tight balance between eat-me (e.g., calreticulin) and do not eat me signals (CD24, CD47, GD2) that determine the susceptibility of target cells to phagocytosis [88]. Independent reports indicate that senescent cells exhibit a concomitant upregulation in the expression of CD47, CD24 and calreticulin [89,90]. It is therefore tempting to speculate (yet it remains to be formally demonstrated) that treatment with monoclonal antibodies targeting CD47 (α CD47), which elicits preclinical and clinical benefits in pathological contexts such as fibrosis would lead to macrophage-dependent senescent cell clearance [91]. A recent report indicates that elevated CD47 expression by senescent cells undermines the ability of macrophages to perform optimal efferocytosis, an event that has been associated with autoimmunity [90].

Non-classical MHC-I molecules may also serve as SAICs. Thus, senescent cells bypass NK cells and CD8⁺ T cells control via the upregulation of the non-classical MHC-I molecule HLA-

E (H2-Qa-1), which acts as a ligand for the inhibitory receptor NKG2A expressed by NK cells and differentiated CD8⁺ T lymphocytes. In co-culture experiments, silencing of HLA-E or NKG2A blockade sensitized senescent fibroblasts to elimination by these immune subtypes [92]. In line with this result, surface expression of the disialylated ganglioside GD3 suppressed NK-mediated cytotoxicity, while the pharmacological neutralization of GD3 by a specific monoclonal antibody rescued NK-dependent clearance of senescent cells in a mouse model of bleomycin-induced pulmonary fibrosis [93]. Recent works consistently report that the senescent cells present in the tissues of naturally aged mice and in specimens from idiopathic fibrosis patients displayed heightened expression of the immune checkpoint molecule PD-L1 [94,95]. Accordingly, treatment of naturally aged mice or animals challenged with a protocol to induce steatohepatitis with anti-PD1 reduced the load of senescent cells in a CD8⁺ T cell dependent manner [94]. Interestingly, a recent work by Chaib and colleagues found elevated expression of PD-L2, but not PD-L1, in multiple cellular models of senescence. Notably, anti-PD-L2 treatment is sufficient to eradicate tumors in which senescence was induced by doxorubicin treatment [96]. Future experiments will clarify whether anti-PD-L2 treatment also diminishes the burden of senescence cell in non-oncological setups.

Taken together, these data suggest that the staggering potential of immune checkpoint blockade therapy to contrast cancer outgrowth might be translated to other pathological settings in which senescence has a defined pathological function. On the one hand, it would be of great relevance to test whether combination approaches based on the targeting of different SAICs would synergize in promoting the elimination of senescent cells by distinct immune subsets and therefore yield superior therapeutic benefits. On the other hand, SAIC expression by senescent cells may serve the purpose to limit pathogenic autoimmunity, thus imposing a note of caution about the safety of such interventions.

From senolysis to immune-senolysis: therapeutic perspectives

In the light of the elevated degree of functional and molecular heterogeneity associated with the senescent state, therapeutic approaches designed to specifically target deleterious senescent subtypes (while sparing their beneficial counterparts) or to eliminate selective senescent cell types present within a tissue are needed. There are now compelling proof-of-concept studies in support of the use of immunotherapy to selectively target senescent cells. Investigators have harnessed the selective (or augmented) expression of cell surface proteins by senescent cells to educate the immune system towards their elimination. As an example, CAR-T cells engineered to recognize urokinase-type plasminogen activator receptor (uPAR) are able to reduce the overall burden of senescent cells in tumor bearing animals or in mice challenged with protocols to induce hepatic fibrosis [97]. Along similar lines, the senescence specific expression of DPP4 can be used to sensitize senescent fibroblasts to NK cell mediated antibody-dependent cellular cytotoxicity [98]. Importantly, this approach has been recently employed to develop ‘senolytic vaccines’ targeting

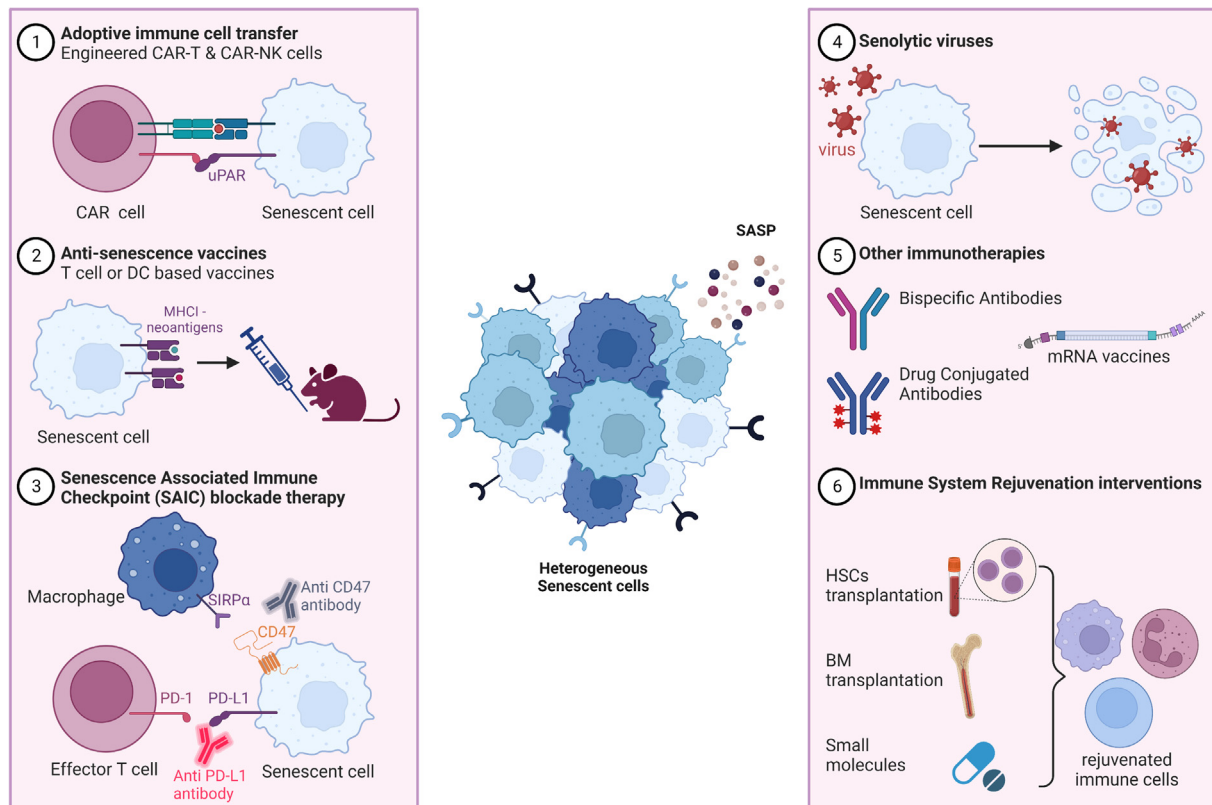


Fig. 2 Approaches of anti-senescence immunotherapy. Currently described (left) and future (right) immune system-based strategies for senescent cell elimination.

Abbreviations: SASP: Senescence Associated Secretory Phenotype; CAR: Chimeric Antigen Receptor; uPAR: Urokinase-type plasminogen activator receptor; DC: Dendritic Cell; MHC: Major Histocompatibility Complex; SIRP α : Signal regulatory protein α ; CD47: Cluster of Differentiation 47; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; HSCs: Hematopoietic Stem Cells; BM: Bone Marrow.

glycoprotein nonmetastatic melanoma protein B (GPNMB) on senescent cells. Notably, immunization of mice against GPNMB reduced the burden of senescent cells, improved the healthspan of naturally aged mice, and prolonged the lifespan of Zmpste24 knockout progeroid mice [99].

Alternative immunotherapy-based interventions to clear out senescent cells can also be conceived (Fig. 2). For example, adoptive immune cell transfer could potentiate the killing of pathogenic senescent cells. As mentioned above, senescent cells exhibit an altered immunopeptidome, resulting in the presentation of unique subsets of antigens that are neither expressed by their normal counterparts nor subjected to thymic selection [76]. While the mechanistic insights underlying this effect remain unknown, such senescence-associated antigenic epitopes may be harnessed for dendritic cell-based vaccination strategies. The goal of such vaccination protocols would be to prime T cells against senescent cells and to instigate durable immunological memory against pathogenic senescent cell variants. In this regard, the use of newly developed animal models [100] – possibly coupled with approaches of genetic or pharmacological senolysis and single cell

technologies – may facilitate the identification of therapeutically relevant senescent-specific antigens.

Conflicts of interest

GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Tollys, and Vascage. GK has been consulting for Reithera. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. GK's wife, Laurence Zitvogel, has held research contracts with 9 Meters Biopharma, Daiichi Sankyo, Pilege, was on the on the Board of Directors of Transgene, is a cofounder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the

microbiota. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim.

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