Reshaping of the tumor microenvironment by cellular senescence: an opportunity for senotherapies

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Keywords: Senescence, Cancer, Tumor microenvironment (TME), Senescence-associated secretory phenotype (SASP), Senolytics

SUMMARY

Cellular senescence is a stress response associated with aging and disease, including cancer. Senescent cells undergo a stable cell cycle arrest, change in morphology, metabolic reprogramming and produce a bioactive secretome termed the senescence-associated secretory phenotype (SASP). In cancer, senescence is an important barrier to tumor progression. Induction of senescence in preneoplastic cells limits cancer initiation and many cancer therapies act in part by inducing senescence in cancer cells. Paradoxically, senescent cells lingering in the tumor microenvironment (TME) can contribute to tumor progression, metastasis and therapy resistance. In this Review we discuss the different types of senescent cells present in the TME and how these senescent cells and their SASP reshape the TME, affect immune responses and influence cancer progression. Furthermore, we will highlight the importance of senotherapies, including senolytic drugs that eliminate senescent cells and impede tumor progression and metastasis by restoring anti-tumor immune responses and influencing the TME.

INTRODUCTION

When cells accumulate detrimental mutations or undergo damage that cannot be repaired, they activate protective mechanisms such as apoptosis or senescence that contribute to maintain tissue homeostasis by avoiding the replication of damaged cells. If these cells escape these protective responses and continue proliferating, they might give rise to tumors [1,2].

Numerous studies have tried to characterize and describe the process of tumor formation over the years and have helped our understanding of cancer biology. Hanahan et al. defined the so-called “hallmarks of cancer” as a set of functional capabilities that a normal cell can acquire during neoplastic growth [2]. Three of these hallmarks are intimately related and will be the focus for us in this review: cellular senescence, immune evasion, and the role of the tumor microenvironment (TME). The TME plays important roles for metastasis dissemination and in the insurgence of resistance to conventional cancer therapies[3,4]. Furthermore, the TME can influence immune responses and trigger immune-suppressive behaviors that facilitate tumor growth. Thus, TME can be considered as an arm that cancer cells use to escape immunosurveillance [4,5].

Cellular senescence, a stress-induced stable growth arrest, is a hallmark of cancer. Oncogene-induced senescence (OIS) imposes a barrier for cancer, limiting progression from pre-malignant to malignant disease. Moreover, many cancer therapies act in part by inducing cancer cell senescence. And while tumors need to escape or avoid senescence to progress, a variety of senescent cells are present in the TME during the lifetime of a tumor. Therefore, senescence seems a ‘friendly’ response: it intrinsically limits cancer and can be evoked by cancer therapies. However, alternatively, through the senescence-associated secretory phenotype (SASP), senescent cells can reprogram the tumor microenvironment and promote more aggressive cancer phenotypes [6–9]. Lingering senescent cells in the TME can also promote tumor growth, induce metastasis and cause therapy resistance. Therefore, how senescence reshapes the TME and what the consequences are for tumor progression is not a straightforward question. This review will first introduce the various components making up the TME, then analyze the effects of senescence response on tumor progression, and how senescence can affect cancer cells and the TME. We will also highlight the importance of targeting senescence thanks to senotherapies. We will mainly focus on the importance of senescent cells elimination through senolytic therapy not only to fight cancer progression but also to eliminate lingering senescent cells in the TME and restore effective immune response or increase the efficacy of immunotherapies.

THE TUMOR MICROENVIRONMENT

The tumor microenvironment (TME) is an ecosystem composed of tumor cells and of several non-tumoral components such as stromal cells, including fibroblasts (often referred as cancer-associated fibroblasts), endothelial cells, immune cells, like neutrophils, macrophages, and lymphocytes and the non-cellular components of the extracellular matrix such as collagen, fibronectin, hyaluronan, laminin, among others [3].

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are one of the main components of the TME. Their abundance is dependent on the tumor type. Several studies show their significant role in pancreatic, colorectal, breast, and prostate cancer. In these cancers, CAFs are responsible for metastasis formation [8,10,11]. CAFs are very heterogenous in nature and originate from different precursors depending on the tumor type. CAFs can derive from stem cells, resident fibroblasts, mesenchymal cells, or bone marrow cells [10,12]. In recent years, single-cell RNA-Seq (scRNA-Seq) has served to better characterize CAFs and have helped to highlight the importance of the TME in influencing the outcome of cancer therapies. scRNA-Seq studies have helped to classify CAFs into different clusters that can be defined by markers, such as alpha-smooth muscle actin (α-SMA), myosin light chain 9 (MYL9), myosin light chain kinase (MYLK), matrix metalloproteinase 2 (MMP2), decorin (DCN) and collagen type I alpha 2 (COL1A2). [3,8,10]. Among these CAF subtypes, there are myofibroblastic CAFs (myCAFs) expressing high levels of collagen isoforms, inflammatory CAFs (iCAFs) expressing inflammatory genes, and mesothelial CAFs (mesCAFs) expressing mesothelial genes [8,10].

CAFs modulate cancer progression and metastasis formation through synthesis and remodeling of the extracellular matrix (ECM) and by secreting growth factors that influence angiogenesis, tumor mechanics, drug access, and therapy responses [11,13]. For example, Zhang et al. demonstrated that CAFs could produce and release different factors, such as NRG1, that can render prostate cancer resistant to castration therapy [11].

CAFs also promote an immunosuppressive environment by excluding or inactivating T cells from the TME [10,12,13]. Bhattacharjee et al., demonstrated that CAFs depletion from the TME did not affect the macrophages and the myeloid compartment but affected the T cell compartment, reducing the numbers of FOXP3+ Treg cells and affecting tumor growth [10]. Because of their involvement in cancer progression, immune evasion, and therapy resistance, CAFs are considered an interesting target to improve cancer therapy and immunotherapy [8,11,13].

Immune cells

Tumors are almost invariably infiltrated by different immune cells. The immune cells that localize inside the tumor are mainly terminally differentiated. Therefore, the role of the peripheral organs like lymph nodes and bone marrow, where hematopoietic progenitors reside is key to replenish the TME of immune cells[14]. These infiltrated immune cells have a crucial role in developing an anti-tumor immune response, but the tumor can subvert them to facilitate tumorigenesis. While their intended activity should be to suppress tumor growth, cancer cells can recruit to the TME different types of immune cells with ability to suppress the anti-tumor immune response [2,14].

Lymphocytes, including natural killer (NK) cells, CD8+ Cytotoxic T cells, and CD4+ helper T (Th) cells, together with pro-inflammatory macrophages (M1) and dendritic cells (DCs) are all involved in anti-tumor immune responses [15,16]. On the other hand, a heterogeneous population of myeloid-derived suppressor cells (MDSCs, also known as activated neutrophils), M2-macrophages and Foxp3+ regulatory T cells (Tregs) suppresses tumor immunity[17–21]. This classification is not strict because some types of immune cells can have a dual role in different contexts. CD4+ helper T cells, for example, can recruit B cells and CD8+ cytotoxic T cells to build an anti-cancer immune response, while CD4+ Treg cells are recruited by cancer cells and can suppress the immune response [15].

MDSCs are a class of activated neutrophils or monocytes that can suppress immune responses in pathologic conditions [20]. MDSCs can suppress the function of T lymphocytes, B lymphocytes, and NK cells, promoting tumor progression and metastasis [21]. Neutrophils are also responsible for metastatic formation in breast cancer, as they escort circulating tumor cells into the blood vessels when they leave the primary tumor site [22]. Both MDSCs and Tregs are associated with poor prognosis in cancer patients [20,23].

Tumor-associated macrophages (TAMs) can be classified into M1-macrophages with a tumor-suppressive activity and M2-macrophages that promote tumor progression [16]. M1 macrophages have an intrinsic function to trap, phagocytize, and lyse tumor cells [24,25]. They promote anti-tumor immunity mediated by CD8 + T cells and NK cells [26]. As a result, M1 macrophages cooperate with the ‘good’ immune cells to induce apoptosis in tumor cells. On the contrary, M2 macrophages are educated by the tumor cells to suppress the anti-tumor immunity and help tumor progression [19,25]. In recent years, the success of checkpoint inhibitors and cellular therapies have highlighted the key role that the immune system plays in controlling tumor growth. Immunotherapy can reactivate anti-cancer immune responses that have been suppressed. Moreover, new therapeutic approaches are aimed to directly kill or re-educate the immune suppressive cells that infiltrate the TME.

Endothelial cells

Endothelial cells and the vasculature system are essential to maintain tumor growth and allow their metastatic dissemination. Cancer cells need nutrients to sustain their growth. Thanks to the expression of angiogenic factors such as vascular endothelial growth factor (VEGF), and hypoxia-inducible factor (HIF), cancer cells promote angiogenesis, the process of formation of new blood vessels, which is critical for tumorigenesis [27,28]. Newly formed blood vessels provide nutrition and oxygen to the tumor, contributing to its growth and development. The endothelium also plays other functions that promote tumor metastasis. This includes being involved in intravasation, allowing invasive cancer cells to translocate into the blood vessel lumen. This phenomenon is an important early stage for cancer metastasis [27,29].

Tumor endothelial cells (TECs) are important components of the TME. TECs are morphologically different from normal endothelial cells (NECs): they show higher fenestrated walls and lose intercellular junction to neighboring cells. They are not organized in a single layer but can grow in a multi-layer fashion [27,30]. TECs have an important role regulating immune cell infiltration inside the tumor, selecting specific immune subsets. TECs favor tumor infiltration of MDCs and T reg, while inhibiting the infiltration of CD8+ effector T cells, DCs, and NK cells. This phenomenon is called “endothelial anergy” and is responsible for promoting an immunosuppressive tumor milieu that helps tumor progression [27,29]. TECs can be responsible for the failure of immunotherapy treatment [31]. Angiogenesis inhibitors were considered as anti-cancer drugs already in the 1970s [32,33]. Most anti-angiogenic agents approved for cancer treatment target the vascular endothelial growth factor (VEGF) or its receptor, as VEGF signaling is the main angiogenesis promotor. Bevacizumab is one of the most used monoclonal antibodies targeting VEGF. These inhibitors normalize the tumor vessels and allow chemotherapeutic drugs and immune cells to penetrate the tumors [29,34,35]. However, early clinical trials of VEGF inhibitors did not show benefits in metastasis formation and survival for cancer patients. Thus, a better therapeutic strategy will be combining these inhibitors with chemotherapy or immunotherapy because targeting TECs will improve chemotherapy and immunotherapy responses [29,36]

SENESCENCE IN CANCER AND THE TUMOR MICROENVIRONMENT

Cellular senescence is a stress response that limits the replication of old, damaged and cancerous cells. Senescent cells undergo a highly stable arrest, and many other phenotypic alterations including morphological changes, metabolic reprogramming and the production of a bioactive secretome termed the senescence-associated secretory phenotype (SASP) [37]. The SASP plays important roles in determining the physiological effects exerted by senescent cells. Senescence was first described more than 60 years ago on cells reaching replicative

exhaustion [38]. Due to cell replication, telomeres become increasingly shorter and eventually, telomere uncapping results in a DNA damage response (DDRs) that activates senescence [1,39]. Besides replicative exhaustion, senescence can be induced by a variety of stresses including oncogenic activation (termed oncogene-induced senescence, OIS) [40], chemo- and radiotherapy (referred as therapy-induced senescence, TIS) [41] pathogens, oxidative stress and other insults. It is not fully understood what determines whether a cell undergoes quiescence, senescence or apoptosis, but it is thought that the duration and strength of the stress might influence cell fate. Interestingly, anti-apoptotic molecules such as BCL2-family members are activated once that a cell commit to senescence[42]. How senescence is induced, which insults cause it and the pathways and phenotypes associated with cellular senescence have been reviewed extensively elsewhere [43,44]. However, importantly, different types of senescent cells emerge and co-exist in the TME during cancer progression (Figure 1).

Preneoplastic senescent cells

Oncogene-induced senescence (OIS) was initially described as the result of ectopic expression of oncogenic $HRAS^{G12V}$ on primary fibroblasts. Oncogenic $HRAS^{G12V}$ expression activates the $p16^{INK4a}$/Rb and the p53/$p21^{CIP1}$ tumor suppressor pathways, resulting in a stable arrest that resembles that observed during replicative senescence [40]. While at that stage it was already hypothesized that OIS could constitute a barrier to tumor progression, it took almost a decade to identify that indeed that was the case. Several pioneering studies showed that senescent cells are present in premalignant lesions such as melanocytic nevi [45], prostate intraepithelial neoplasias (PIN) [6], lymphomas [46], pancreatic intraepithelial neoplasia (PanIN) [47], lung adenomas[48] and pituitary tumors [49]. Since then, the paradigm that premalignant lesions are enriched in senescent cells has been extended with similar observations in the precursor lesions to hepatocellular carcinoma, breast tumors or neurofibromas [50,51].

While initially described in response to the expression of oncogenic $HRAS^{G12V}$ [40], other oncogenes such as Braf, Myc or E2F3 or β-Catenin [52–55] also cause OIS. A similar response is also observed in response to loss of tumor suppressors, such as PTEN [6,56] or NF1 [51,57]. For example, loss of Pten in prostate cancer triggers a senescence response termed Pten-loss-induced cellular senescence (PICS) [56]. Overall, induction of senescence in premalignant lesions limits cancer progression by a combination of a stable growth arrest and activation of immune surveillance [58]. Moreover, restoration of p53 in sarcomas [59] and liver carcinomas [60] causes their regression due to senescence induction and the concomitant activation of an anti-tumor immune response (Figure 1).

Cells undergoing therapy-induced senescence

Most cancer therapies can trigger senescence. These include radiotherapies, chemotherapies and targeted therapies, such as CDK4/6 inhibitors. Different drugs can engage senescence in different ways: such as inducing DNA damage (e.g., doxorubicin, etoposide, or platinum-based compounds), reacting with DNA (e.g., alkylating agents), inhibiting cell cycle (e.g. palbociclib) or directly inhibiting proliferation (e.g., docetaxel and paclitaxel) [1,41,61].In general, tumor cell senescence upon therapy treatment (often referred as therapy-induced senescence, TIS), contributes to the outcome of cancer therapies [41] (Figure 1).

While most normal senescent cells are diploid, induction of TIS in many cancer cells coexists with induction of polyploidy. It has been argued that polyploidy might contribute to some of the characteristics of senescence[62]. TIS results in an increase in polyploid senescent cells in several types of cancer, such as NSCLC and Burkitt lymphoma [63,64]. Polyploidy can represent a survival mechanism for cells to avoid apoptosis or mitotic catastrophe upon genotoxic stress[65,66].

Indeed, induction of senescence can predict the outcome of therapies in patients with metastatic colorectal cancer [67]. However, lingering tumor cells can drive different aspects of tumor progression. In addition, induction of senescence by cancer therapies is not limited to the tumor itself. For example, radiotherapies, despite been targeted to an area, can cause senescence in the TME. These senescent cells are known to mediate radiation-induced fibrosis, that is a frequent complication of radiotherapy of lung tumors [68]. Similarly, treatment with the CDK4/6 inhibitor palbociclib can cause stromal senescence that fuels tumor growth [69] .

Paracrine senescence in the TME

Senescent cells influence their neighboring cells through the SASP. Amongst the different effects that the SASP mediates, it is the induction of paracrine or bystander senescence [70]. Several components of the SASP, including TGF-β, IL-1 or CCL2 can cause paracrine senescence[70,71]. Moreover, ROS propagating via gap junctions [72] and extracellular vesicles [73] also can cause paracrine senescence. Examples of paracrine senescence can be observed in the tumor microenvironment: senescent preneoplastic hepatocytes are surrounded by immune cells, some of which stain positive for senescence markers; and different stromal cells found in the proximity to papilloma lesions also express senescent markers [70]. Non-senescent cells in the TME (such as tumor or immune cells) also can induce senescence on their neighbors. Tumors can induce senescence in their stroma as shown taking advantage of a p16-luciferase reporter mice [74]. Conversely T-helper-1 cells infiltrating the TME can induce senescence in the tumor in a way dependent of TNF and IFN-γ [75] (Figure 1).

Senescent cells accumulating with aging

Senescent cells accumulate and contribute to ageing. The expression of $p16^{INK4a}$ accumulates with ageing in multiple tissues [76] and selective ablation of $p16^{INK4a}$-positive senescent cells, taking advantage of INK-ATTAC mice has shown how senescent cells contribute to ageing [77]. The incidence of cancer also increases with age [78], and indeed the extension of lifespan observed upon depletion of senescent cells in the INK-ATTAC mice can be attributed in part to a delayed cancer onset [77]. Aging influences the composition of the TME (Figure 1), which consequently can promote tumor growth and metastasis dissemination [79]. While age-related accumulation of senescent cells in the TME cannot by itself explain the increase in cancer during ageing, it has been shown that stromal senescence (which increases with age) accelerates tumorigenesis by establishing an immunosuppressive environment [80].

An important hallmark of aging is the increase of systemic low-grade chronic inflammation known as "inflammaging" [81]. This persistent inflammation can lead to tissue disruption, cancer promotion and progression [79,82]. Cellular senescence is a key contributor to inflammaging, as elimination of senescent cells reduce the levels of inflammatory cytokines during aging [77]. Inflammaging is also responsible for MDSCs recruitment and, consequently of T cells immunosuppression in several types of cancers [79,83].

Heterogeneity of cellular senescence

In addition to all the different types of senescent cells coexisting in the TME, senescence itself is a highly heterogeneous response. Heterogeneity impacts both the role that senescent cells play *in vivo* and our ability to identify them [84]. The senescence transcriptome is a temporally dynamic programme that also depends on the type of cell and how senescence is induced [85]. Recently Jochems et al. characterized senescence heterogeneity in a panel of 13 cancer cells treated with two different senescence inducers, alisertib (Aurora kinase A inhibitor) and etoposide (topoisomerase II inhibitor) [86]. They showed that the major source of transcriptional heterogeneity is the cell type. Importantly, while p53 wild-type cell lines showed senescence arrest in the G1 phase of the cell cycle, while the p53-mutant ones were not arrested in a specific phase of the cell cycle [86].The heterogeneity of senescence is reflected in the SASP [87]. SASP composition changes with time [88] and reflects the basal levels of secreted factors expressed before senescence induction[85,86]. The relevance of SASP heterogeneity is underscored by the fact that different SASP components mediate different functions. While often SASP function is context-dependent, the heterogeneity and composition of the SASP would also influence their effects. In this context, the trigger and nature of the cells undergoing

senescence (e.g. stromal, endothelial or epithelial) will dictate the effect that the SASP will have in the TME [89]. Senescence fibroblasts secrete chemokines such as IL6 or CXCL1 and CXCL2 that can stimulate a pro-tumorigenic immune response and therapy resistance[79,90]. NOTCH1 can prevent a pro-inflammatory SASP secretion in fibroblast, while the JAK-STAT pathway regulates the secretion of an immunosuppressive SASP in prostate cancer cells [88,91]. The heterogeneity of senescence complicates the identification of senescent cells. While some characteristic such as their enlarged morphology and increase in SA-β-galactosidase activity can serve to identify senescent cells, they are not unequivocal. For example, macrophages can show high levels of β-galactosidase activity. Given the potential of senotherapies, it is crucial to develop non-invasive methods to measure senescence in patients [1]. While their heterogeneity means it is difficult to identify senescent cells, different methods, such as algorithms like SENCAN or gene signatures of senescence can be developed for their identification [85,86].

HOW SENESCENT CELLS INFLUENCE THE TME

Induction of senescence during tumorigenesis has cell intrinsic effects (such as the growth arrest, essential for their anti-tumor effects), but many of their pathophysiological effects are mediated in a paracrine manner. Paradoxically, senescence can have both positive and negative effects on tumor progression (Figure 2). Secreted factors are key signals that regulate the epigenetic mechanisms involved in tumor-stroma crosstalk [92]. Similarly, senescent cells communicate and influence their neighbors via secreted factors (the SASP) and cell-cell interactions. This is true of all senescent cells, especially in the context of tumorigenesis. Through these paracrine effects, senescence can directly affect the tumor properties, facilitating tumor resistance, metastasis or growth. Pro-senescence therapies can have detrimental effects on healthy tissues, provoking senescence in non-cancerous cells [61,93]. Chemotherapies can provoke in patients important side effects such as cardiac dysfunction, inflammation, or immunosuppression [61,93]. While such effects happen outside the TME, senescent cells can also influence the tumor itself and the TME, in turn having a profound impact on tumorigenesis. For example, they affect the immune system in multiple ways for example, by activating the immune surveillance response or inducing an immunosuppressive microenvironment. Senescent cells can also educate CAFs or regulate tumor vascularization.

Effects of the SASP on tumor cells

Senescent cells can remodel the TME and dictate its composition, while also directly influencing the behavior of the tumor itself. The initial observation of senescent cell cross-talk with cancer cells was derived from co-culture and xenograft models showing that senescent fibroblasts stimulate tumorigenesis or malignant and premalignant cells [94]. Indeed, eliminating senescent fibroblasts or inhibiting their pro-inflammatory SASP limits tumor growth in xenograft models [95–97]. The nature of the senescent cells that promote tumor growth can be diverse. In a model of obesity-induced liver cancer, metabolites produced by the gut microbiome cause senescence of hepatic stellate cells (HSCs), and the proinflammatory SASP of HSCs contributes to hepatocellular carcinoma (HCC) development [98]. In adamantinomatous craniopharyngioma (ACP), a type of pediatric brain cancer, β-catenin-positive premalignant lesions are senescent and promote tumor growth in a paracrine fashion[99]. The SASP can also influence other tumor promoting characteristics, such as causing epithelial-to-mesenchymal transition (EMT) [90] and is responsible for the insurgence of therapy resistance, tumor progression, and metastatic formation [7,93]. Overall, the SASP plays multiple, context-dependent roles that not only influence directly the fate of premalignant and malignant cells but also shape the TME and influence immune responses [100] (Figure 2).

Senescence influences immune responses in the TME

Induction of senescence, either by oncogenic activation in preneoplastic lesions or as response to cancer therapies has anti-tumor effects (Figure 2). Besides becoming arrested, premalignant or cancerous senescent cells activate immune surveillance via the SASP. Tumor liver cells in which p53 expression is reactivated are cleared in a way dependent of the recruitment of different immune cells (including NK cells, neutrophils and macrophages) [60], and pro-senescence therapies (such as the combination of Palbociclib and trametinib, P+T) cause a NK-mediated elimination of lung tumors[101]. Interestingly, the immune responses elicited by senescent cells are context-dependent: PDACs treated with P+T do not attract NK cells, but instead recruit PDL-1+ CD8+ T cells. Combination with checkpoint inhibitors can reactivate the anti-tumor potential of these T cells [102]. In ovarian cancer, induction of senescence with platinum-based therapies (cisplatin) can enrich the TME in T cells and NK cells, activating an anti-tumor immune response. This happens preferentially in some genetic backgrounds, such as homologous recombination (HR)-deficient ovarian cancer, where cisplatin induces a stronger senescence response [103].

Preneoplastic hepatocytes expressing oncogenic $Nras^{G12V}$ are cleared by CD4 T cells assisted by monocyte/macrophages [58]. Secretion of several SASP components, including IL1 or CCL2 is needed for the recruitment of the immune cells [70]. Indeed, CXCL14 secretion by Ras-

expressing hepatocytes occurs before they become fully senescent and serves to attract macrophages [104]. The SASP produced by senescent hepatocytes or stellate cells polarize the macrophages towards an M1 phenotype and helps to eliminate the senescent cells [104,105]. On the other hand, p53-deficient stellate cells produce a SASP that promotes M2 polarization and tumor growth [105]. The SASP can contribute to create an immunosuppressive microenvironment in other ways. For example, if the senescent program is bypassed or senescent cells persist during liver tumorigenesis, the SASP can recruit a population of CCR2+ immature myeloid cells (iMCs) that impedes NK action [100,106]. iMCs also infiltrate PTEN-null prostate tumors and antagonize senescence through secretion of interleukin-1 receptor antagonist (IL-1RA) [18,100].

Immune cells infiltrating the TME can also undergo senescence. Tregs or cancer cells can induce senescence in effector T cells [107], impairing their ability to eliminate their targets [108]. Senescent T cells present low levels of the co-stimulatory molecules CD27 and CD28, and they do not proliferate upon TLR8 stimulation [109]. They release a pro-inflammatory SASP that can create an immunosuppressive TME by recruiting myeloid cells and T reg cells [107,110]. Senescent T cells are unable to eliminate tumor cells, and therefore by inducing senescence in T cells, the tumors can avoid immunosurveillance [107,108,111].

Induction of senescence in CAFs

Cancer-associated fibroblasts (CAFs) are a heterogeneous population of fibroblasts. Many subtypes of CAFs exist, including a subpopulation of p16+, senescent CAFs. In head and neck (HNSCC) and esophageal (EAC) cancers, most of these p16+ CAFs express SMA and display features of myofibroblasts [112]. The presence of p16+ CAFs with features of myofibroblasts is a prognostic factor in these cancers [112]. CAFs can also develop a senescence response following TIS or radiation therapy in the primary tumor. Nicolas et al.[8] demonstrated that in rectal cancer patient-derived organoids, resistance to conventional radiotherapy is due to a p53-dependent senescence response in CAFs. Senescent CAFs release a SASP that promotes the survival of tumor cells, exclusion of cytotoxic T cells from the TME, and eventually causes therapy resistance [8,113].

Senescence influences TME vascularization

Senescence induction in the TME can also influence the endothelial compartment. By secreting vascular endothelial growth factor (VEGF), senescent cells stimulate endothelial cells and increase tumor vascularization [114]. While increased angiogenesis promotes tumor growth, there are instances where senescent cell signaling to the endothelium mayimprove anti-tumor responses. For example, treatment of pancreatic tumors with palbociclib and

tremetinib, causes senescence and induces vasculature remodeling and neoangiogenesis. This improves the access of chemotherapeutic agents to the tumor and facilitates immune infiltration [102] Similarly, immune surveillance of senescent preneoplastic hepatocytes is facilitated by SASP-mediated NF-kB activation in endothelial cells. This event is necessary for CD4+ and CD8+ lymphocytes adherence and trans-endothelial migration. In this case, SASP signaling to the endothelial compartment is essential for lymphocyte recruitment as part of an anti-tumor immune response [115] (Figure 2).

Senescence and tissue regeneration and changes in stemness

Tissue damage (such as damage associated with fibrosis) is a cause of senescence. Senescent cells accumulating during aging can favor regeneration via SASP-mediated enhancement of cellular reprogramming [116]. An increase in regeneration is observed in damaged tissues. Induction of senescence in response to lung fibrosis [116] or muscle injury [117] favors tissue regeneration in an IL-6-dependent fashion.

This relationship between increased senescence and enhanced stemness is also observed in cancerous tissues. For example, an increase in activation of stem cells markers is observed in the proximity to preneoplastic senescent hepatocytes and senescent papillomas [118]. Moreover, Milanovic et al. [119] demonstrated that stemness and senescence are co-regulated by overlapping signaling networks. Cells that undergo senescence after oncogene activation or in response to chemotherapy have increased plasticity and express a stem cell signature [119,]. In p53-null lung cancer context, it has been shown that chemotherapy induces an arrest in G2-M phase of the cell cycle Cdc2/Cdk1-dependent, called accelerated cellular senescence (ACS). A subset of these senescent cells can re-enter the cell cycle thanks to the overexpression of Cyclin B1 [120]. Milanovic et al suggest that the increased aggressiveness of those cells that have escaped senescence is linked to the co-regulation of stemness [117].

More importantly, cancerous cells that have previously undergone senescence behave more aggressively and displayed a higher tumor potential when compared to those which never underwent senescence [119]. Wnt is a mediator of this senescence-associated stemness, and senescent cells that re-entered cell cycle are not only more aggressive, but also display therapeutic resistance and increased development of metastasis [119,121] (Figure 2)

SENOTHERAPIES FOR CANCER: TARGETING THE TME

Most cancer therapies act in part by inducing senescence in the tumor. While this senescence induction has an initial tumor suppressive role, it also shapes an immunosuppressive TME and can act off-target to cause side effects [93]. In addition, in the long-term lingering senescent

cells promote many detrimental actions that contribute to tumor progression and therapy resistance. Therefore, targeting senescent cells with so-called senotherapies (summarized in Table 1) is starting to be postulated as a novel anti-cancer therapy. Amongst senotherapies, a new strategy, termed 'one-two punch' has been proposed to enhance the positive effects of senescence induction by cancer therapies while protecting from their detrimental consequences [1].

The therapy consists of a sequential treatment with senescence-induced cancer therapies, followed by senolytic drugs, that selectively kill the senescent tumor cells. Different senolytic drugs have been described, including BCL2-family inhibitors (such as ABT-263 [122,123] or ABT-737 [42]), the combination of the tyrosine kinase inhibitor dasatinib and the flavonoid quercetin (D+Q) [124], cardiac glycosides [125,126] and many others [1]. Several of these senolytic drugs have proven successful in "one-two punch" combination therapies against cancer. For example, lung and breast cancer cells treated with topoisomerase inhibitors (such as etoposide or doxorubicin), undergo senescence, and sequential treatment with the senolytic ABT-263 further impaired tumor growth [127]. Prostate cancer (PCa) cells also became senescent after taxane treatment (docetaxel), and sequential treatment with ABT-263 kills senescent cells, decreasing tumor growth [7]. Recently, it has been discovered that the pro-survival gene MCL-1 is upregulated in senescent PCa cells and treatment with an MCL-1 inhibitor reduces tumor growth and metastatic onset in PCa models [128].

The effectiveness of other combinations of senescence inducers and senolytics has also been proven. In liver cancer cells, CDC7 inhibitors are used to induce senescence and can be combined with an mTOR inhibitor as senolytic to treat HCC[129]. Furthermore, approaches other than small molecules have been developed to kill senescent cancer cells. Amor et al. described that CAR T cells can be used as senolytics. They identified the urokinase-type plasminogen activator receptor (uPAR) as a cell-surface protein characteristic of senescent cells. CAR T cells against uPAR can remove senescent cancer cells both *in vitro* and *in vivo* [130]. Furthermore, Yoshida et al. developed a modified vaccine to kill senescent T cell expressing CD153 on the surface. They showed that the killing of senescent T cells ameliorates obesity-related metabolic disorders [131,132]. The specific combination to be used depends on the nature of the tumor, but the overall concept of "one-two punch" therapies is now ready to be tested in the clinic (Figure 3). Indeed, some senolytics such as Navitoclax are already in trials in the context of a 'one-two punch' strategy, to target solid tumors [133]. Senolytics are also effective limiting cancer growth beyond their use in 'one-two punch' strategies. In pancreatic intraepithelial neoplasia (PanIN), senescent cells induce growth and progression thanks to Cox-2 activity. Senolytic treatment with ABT-737, another BCL-2 inhibitor, targets this Cox-2 expressing senescent cells, reducing tumor growth [47].

An alternative strategy to senolytics is to potentiate the immune mediated elimination of senescent cells. This can be achieved using CAR-T cells directed against senescent antigens such as uPAR [130], by invariant natural killer T cell activation [134] or using checkpoint inhibitors such as anti PD-1 antibodies [135]. Finally, senomorphic drugs, that do not kill senescent cells but rather modulate the SASP to revert the phenotypes associated with senescence[136] also exist (Table 1). As the SASP mediate most of the detrimental effects associated with senescence, senomorphic drugs can be beneficial in situations where the elimination of senescent cells could compromise tissue integrity. Senomorphic drugs include mTOR inhibitors such as rapamycin [95,96], and inhibitors targeting p38 MAPK [137], NF-κB [138], JAK2[91]or blocking IL-1[136,139].

Effects of senotherapies on the senescent TME and beyond

The main goal of 'one-two punch' approaches is to eliminate the tumor, going further than the cytostatic effects of senescence. There are other added benefits associated with the elimination of senescent cells in the tumor, TME and beyond. Eliminating senescent cells *per se* that otherwise would linger in the tumor has the advantage of targeting the many pro-tumorigenic effects that senescent cells mediate. For example, Demaria et al. showed that the elimination of senescent cells in the context of tumorigenesis reduced inflammation and cancer recurrence [93]. This is likely a combination of interfering with the many SASP-mediated effects that fuel tumor growth, metastasis and therapy resistance. Moreover, depleting senescent cells has also the effect of diminishing many side-effects of chemotherapies, such as bone marrow suppression, cardiac dysfunction or decrease in physical fitness [93]. Such effects are likely the result of eliminating senescent cells that arise far from the tumor or the TME itself, in normal tissues such as in the heart or bone marrow. Oncogene-induced senescence limits the progression of premalignant lesions. Despite the effectiveness of OIS as a tumor suppressor barrier, cells escape OIS to give rise to tumors. Senolytic drugs have been proven effective in eliminating preneoplastic senescent cells. For example, treatment with ouabain reduced the presence of preneoplastic $Nras^{G12V}$-expressing hepatocytes in a model of liver cancer initaiation [126]. Therefore, senolytic therapies could provide the added benefit of reducing cancer initiation rates, or even be used as a prophylactic therapy.

Nicolas et al. showed that coupling radiation therapy with venetoclax, a well-known senolytic drug, reduces tumor growth and liver metastasis formation in rectal cancer. The benefit was associated with the elimination of senescent iCAFs that characterize the rectal cancer TME. Targeting these components of the TME helps to avoid the insurgence of radiotherapy resistance [8]. In agreement with that model, elimination of senescent fibroblast using the senolytic procyanidin C1 or targeting their pro-inflammatory SASP with mTOR inhibitors,

reduces tumor growth in xenograft models [95-97]. Further proof that the effect of senolytic therapies can go beyond directly targeting the tumor comes from recent work from Troiani et al. They demonstrated that targeting senescent prostate cancer cells with MCL-1 inhibitors, does not only suppress tumor growth but also helps to prevent metastasis and reactivates the tumor immune response decreasing the infiltration of MDSCs and increasing T cell activation [128]. Senomorphic drugs such as JAK2 inhibitors can also be used to 'reprogram' the SASP on Pten-null PCa tumors and enhance chemotherapy efficiency by restoring anti-cancer immune responses [91].

Caveats to the use of senolytic therapies

Early studies suggested that delayed wound healing was the only side-effect associated with senolysis [77].The lack of overt side effects, together with benefits associated with intermittent use were attractive and helped to expand the potential therapeutic uses of senolytic drugs. However, our current knowledge suggests that removal of senescent cells in some contexts could be detrimental, and we need to exert caution. Certain cell types such as M2 macrophages acquire senescent-like properties (such as expressing $p16^{INK4a}$ or stain positive for senescence-associated β-galactosidase activity) [140]. The consequences associated with killing these or other subtype of macrophages might be context. Similarly, megakaryocyte maturation [141] or mesenchymal/progenitor stem cells (MPSCs) in late puberty [142] undergo senescence, and ablating these cells might have unintended consequences because, in this context, senescence is a physiological response to interrupt bone growth [142]. While senescent cells are often a small percentage of those present in a tissue, that might not be the case in some elderly individuals and senescent cell ablation might affect tissue integrity. Indeed, studies carried out using a mouse model reporter for senescence have identified that when liver sinusoidal endothelial cells (LSECs) undergo senescence, their ablation results in vascular leakage and fibrosis induction [143,144]. Senomorphics might be a good alternative to senolytics in some of these situations as they would avoid the elimination of senescent cells that might be required for maintaining tissue integrity, while targeting the negative effects associated with a proinflammatory, profibrotic SASP.

CONCLUSIONS AND PERSPECTIVES

While the genetic composition of tumors, with their inherent heterogeneity and evolution is the driving force of cancer, tumors do not exist in isolation and targeting the TME can be a powerful weapon in the anticancer arsenal. In this regard, therapies that reshape anti-tumor immune

responses have recently revolutionized cancer treatment [145]. CAFs are another key target to develop and identify novel cancer therapies [146]. Recently, we have also come to the realization that many different senescent cells coexist in the TME. Senescent cells have proven a novel therapeutic target relevant to many age-related diseases, including cancer [77].

To be able to target senescent cells to influence cancer and the TME, a few challenges remain, namely, (i) we need to discover the different types of senescent cells present in the TME, (ii) understand the roles of those different senescent cells, (iii) identify what is the best way to target (some of) those senescent cells and (iv) be able to monitor senescence in the tumor to stratify patients and follow up the efficacy of senotherapies.

The use of single cell RNA-Seq combined with spatial transcriptomics should contribute to give us a clearer idea of the distribution of senescent cells in the TME and the effects caused by their depletion. For example, a recent study took advantage of scRNA-Seq to identify senescent cells during pancreatic tumorigenesis, discovering a subtype of COX2-positive PanIN cells relevant for progression to PDAC [47]. Similar studies might serve to identify senescent cells present in the TME of different types of cancer as a pre-requisite to define their function.

Often, the persistence and buildup of senescent cells in the tissue is what turns a positive response (acute senescence) into a detrimental response. An acute senescence response limits the persistence of damaged cells cells in the tissue. However, when senescent cells are not cleared by the immune system, they accumulate and their SASP contributes to disrupt tissue homeostasis. Therefore, understanding how senescent cells avoid immune surveillance will be essential to find additional ways to both understand their roles and modulate their effects (Figure 2). One of the potential benefits of eliminating senescent cells in the context of cancer and the TME is that a single therapeutic agent could hit different targets (in the tumor, at the TME and even reduce some of the side effects of cancer therapies) (Figure 3). Broad spectrum senolytics could be attractive to use in cancer therapies. A potential drawback is that there might be senescent cells targeted by senolytics that we do not want to eliminate. For example, recently it has been shown that the elimination of senescent liver sinusoidal endothelial cells (LSECs) can cause fibrosis [143]. Therefore, senolytics that specifically ablates a subpopulation of senescent cells might have to be used in certain circumstances. To be able to use senolytic therapies effetcively, we need to be able to monitor senescence in the tumors and for this, advances in imaging technologies to identify senescence longitudinally in tumors and the identification of biomarkers of senescence is key [147].

Overall, senotherapies represent a promising new therapeutic strategy in cancer patients, as they can act both on cancer cells and non-cancerous components of the TME. This pleiotropic action can enhance the success of combining conventional cancer therapies (that themselves

cause senescence) with senolytics. To fully deploy these approaches, we need to better catalogue the repertoire of senescent cells in the TME and understand their crosstalk with the tumor and the TME itself.

ACKNOWLEDGEMENTS

Figures were created using Biorender.com. For the purpose of open access, the author has applied a Creative Commons Attribution (CC BY) license. This work was supported by a core grant from MRC (MC_U120085810) and a grant from CRUK (C15075/A28647) to J. G.

DECLARATION OF INTERESTS

J.G. has acted as a consultant for Unity Biotechnology, Geras Bio, Myricx Pharma and Merck KGaA. Pfizer and Unity Biotechnology have funded research in J.G.'s lab. J.G. has share options of Geras Bio and Myricx Phrama. J.G. is a named inventor in MRC and Imperial College patents related to senolytic therapies.

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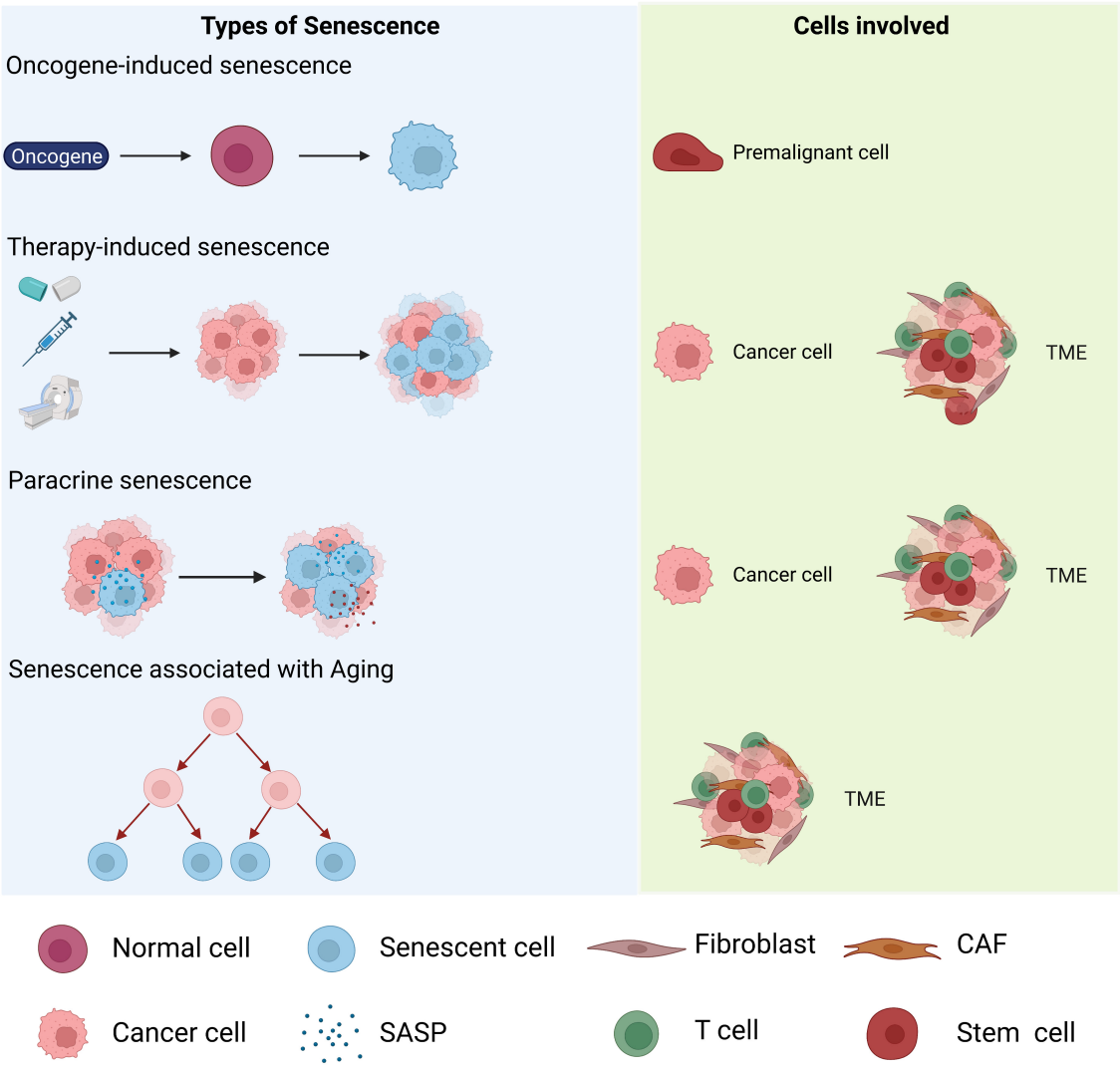


Figure 1. Types of senescent cells co-existing in the Tumor microenvironment.
Senescence can be induced by different stimuli: oncogene activation (oncogene-induced senescence, OIS), chemotherapy or radiation therapy (TIS), paracrine causes and physiological aging. Factors secreted by senescent cells (SASP) or sometimes by non-senescent cells can also induce senescence in neighboring cells in a paracrine manner. What cell type is affected? OIS affects premalignant cells. TIS and paracrine senescence can affect cancer cells and the TME, while aging-associated senescence affects many types of cells including those present in the TME.

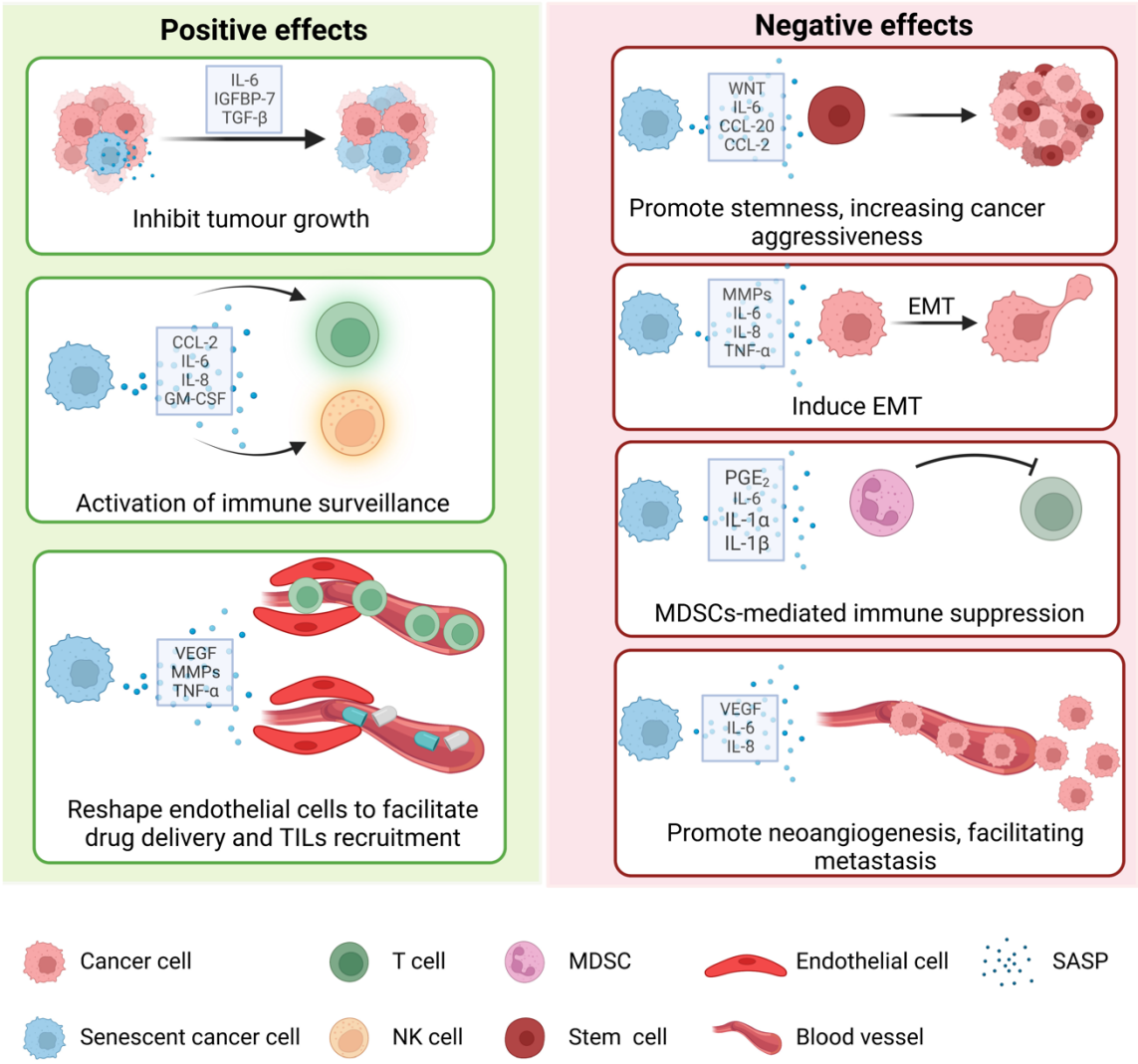


Figure 2. Positive and detrimental effects of senescence in the tumor and the TME

On the one hand, induction of senescence in the tumor limits cancer progression in several ways and can be regarded as a positive effect (see panels on the left side): both by limiting tumor initiation and growth but also by initiating immune surveillance and reshaping the TME to facilitate immune responses and drug delivery. On the other hand, there are negative effects (see panels on the right) . Senescent cells persisting in the TME contribute to tumorigenesis by promoting tumor growth and increasing the aggressiveness of the tumor in different ways: inducing epithelial to mesenchymal transition (EMT) that facilitate metastasis dissemination; releasing SASP factors that facilitate MDSCs recruitment and promote a tumor suppressive TME or inducing neoangiogenesis that facilitates tumor growth and metastasis formation.

<ALG to authors: the last right bottom panel seems less clearly explained to me, please add more details to the legend for this panel.>

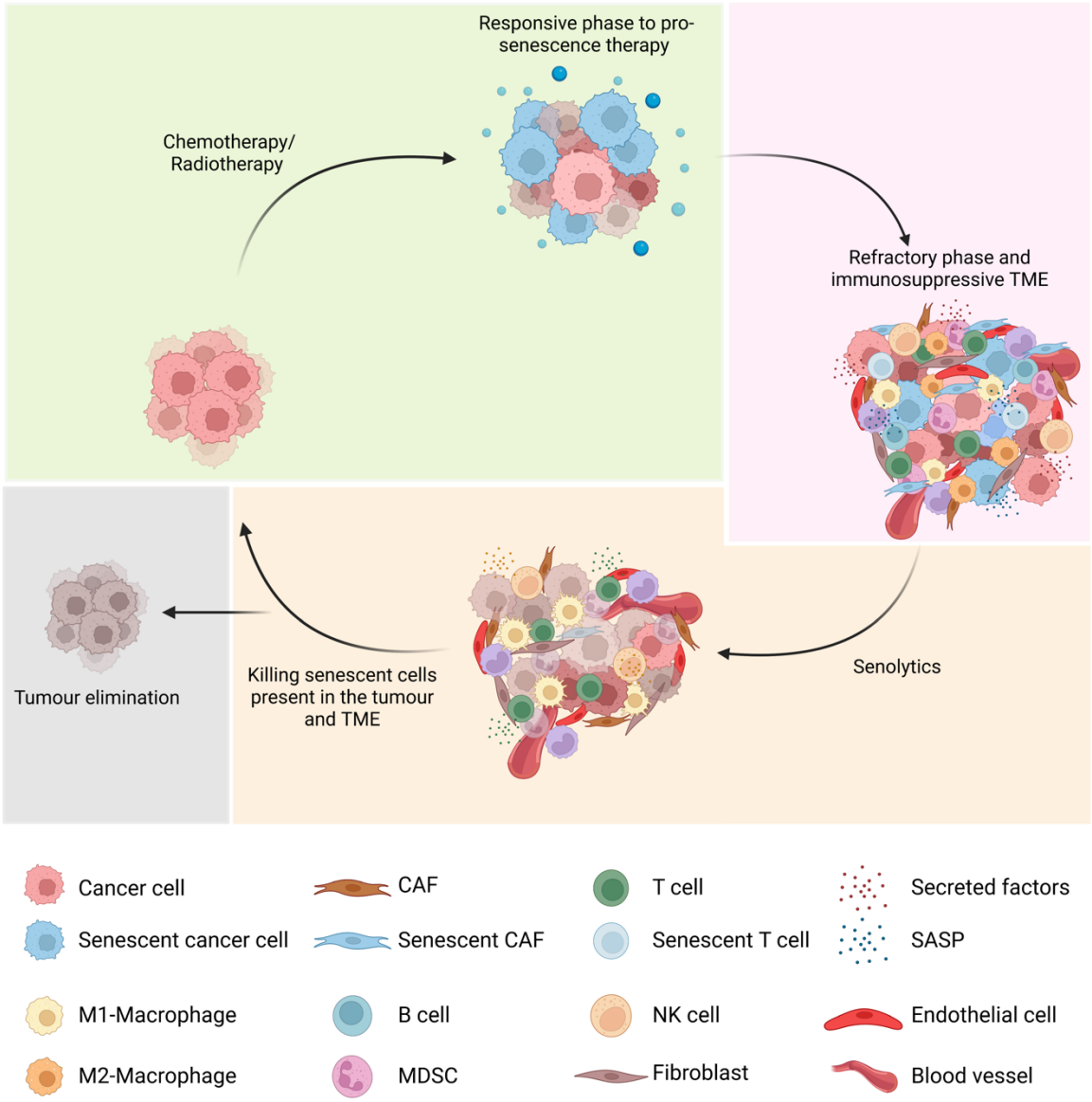


Figure 3. Senolytic therapies can target both the tumor and the TME

Conventional cancer therapies act in part by inducing senescence in the tumor (see panel in green). This limits tumor growth and mounts an immune surveillance response. However, in the long term, lingering senescent cells that are not eliminated by the immune system can promote tumor growth and conversely contribute to create an immunosuppressive TME (in pink). A two-punch approach is therefore needed: treatment with senolytics following senescence-inducing cancer therapies will kill senescent cells (not only in the tumor, but also in the TME), reducing tumor size and decreasing the formation of metastasis (orange panel). While this combinatorial treatment could itself result in tumor elimination, more likely sequential cycles of senescent-inducing therapies and senolytic treatments might be required (grey panel).

Table 1. Examples of different types of senotherapies and their targets.

Type of senotherapy	Drugs	Targets
Senolytic drugs	ABT-263 (Zhu et al.[122], Chang et al.[123]) ABT-737 (Yosef et al.[42])	Bcl-2, Bcl-XL
	S63845 (Troiani et al.[128])	Mcl-1
	Dasatinib + Quercetin (Zhu et al.[124])	Tyrosine kinases + multiple targets
	AZD2014 and AZD8055 (Wang et al.[129])	mTOR
	Cardiac glycosides (Triana-Martinez et al.[125], Guerrero et al.[126])	Na^+/K^+ ATPase
Immune modulators	uPAR (CAR)T cells (Amor et al. [130])	uPAR
	CD153 peptide conjugate (Yoshida et al.[132])	CD153+ T cells
	αPD1 (Wang et al.[135])	PDL-1/PD-1
	α-GalCer (Arora et al.[134])	iNKT cells
Senomorphic drugs	Rapamycin (Herranz et al.[95], Laberge et al. [96])	mTOR
	NF-κB inhibitors (Acosta et al.[138])	IKK
	SB203580 (Freund et al.[137])	p38 MAPK
	Ruxolitinib (Toso et al.[91])	JAK2