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**CELLULAR SENESENCE IN IMMUNE  
AND CANCER CELLS AS MECHANISM  
FOR TREATMENT RESISTANCE IN DIF-  
FUSE GLIOMAS**

Faculty of Medicine and Health Technology (MET)  
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# ABSTRACT

Anni Inkinen: Cellular Senescence in Immune and Cancer Cells as Mechanism for Treatment Resistance in Diffuse Gliomas  
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Gliomas are the most common malignant primary brain tumors, often associated with poor prognosis. Most of these are diffuse, meaning that they infiltrate brain tissue and cannot be completely removed surgically. Glioblastoma is the most aggressive form of diffuse glioma, highly resistant to treatments and prone to relapse. Conventional treatment methods include partial surgical resection followed by chemotherapy with Temozolomide and radiotherapy, which are noted to induce senescence in glioma cells and in normal cells in the tumor microenvironment.

Senescence is normally an irreversible and stable cell cycle arrest in either the G1 or G2 phase, where cells do not proliferate in response to mitogenic signals. However, senescent cells remain metabolically active and produce a senescence-associated secretory phenotype (SASP) that leads to the secretion of various chemicals, such as cytokines, chemokines, growth factors, and proteases. Senescence can be induced by several factors, such as DNA damage, oncogene activation and therapeutic agents. These factors often lead to the activation of tumor suppressor pathways, such as p53/p21 and/or p16/pRb. Senescence can be divided into three categories according to the inducing factor: replicative, oncogene-induced, and therapy-induced senescence.

Senescence and its associated phenotype have a dual role in the context of cancer. Intrinsically, senescence has an anti-tumorigenic role by resulting in cell cycle arrest that inhibits uncontrolled proliferation of cancer cells. Additionally, some of the SASP factors can lead to immune surveillance and clearance of senescent cells by recruiting immune cells, such as natural killer cells and T cells. Senescence can also have pro-tumorigenic role in cases of senescence escape or evasion. Some SASP factors can mediate tumor growth by altering the tumor microenvironment. They can recruit immunosuppressive cells and affect their polarization. They can also remodel the extracellular matrix, develop metastatic niches, promote angiogenesis, and induce epithelial-to-mesenchymal transition, all of which are important features of cancer progression.

Currently, cellular senescence is under research because of its known role in age-related pathologies, such as cancer. Understanding how senescence could lead to treatment resistance in diffuse gliomas could help develop new, more effective therapy approaches. Recently, it has been under active investigation whether senescence could be utilized for cancer treatment through senescence-targeting drugs, such as senolytics and senostatics. However, there is an urgent need to find reliable and reproducible markers that specifically target senescent cells and/or the senescent phenotype to identify senescent cells and monitor treatment responses.

Keywords: cancer, diffuse glioma, glioblastoma, treatment resistance, cellular senescence, immune cell senescence, cancer cell senescence, SASP, senolytics, senescence markers

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# PREFACE

This thesis has been written as a literature review at the Faculty of Medicine and Health technology, Tampere University, as a part of the Bachelor of Science degree. I would like to thank Professor Katri Lindfors for making my thesis topic possible. In addition, I would like to thank my supervisors Doctoral Researcher Aliisa Tiihonen and University Lecturer Kirsi Rautajoki for providing my thesis topic and for invaluable supervision and support.

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## ABBREVIATIONS

<b>ECM</b>	extracellular matrix
<b>SASP</b>	senescence-associated secretory phenotype
<b>WHO</b>	World Health Organization
<b>IDH</b>	isocitrate dehydrogenase
<b>MGMT</b>	O6 -methylguanine-DNA methyltransferase
<b>Bcl-2</b>	B cell lymphoma 2 protein family
<b>BAX</b>	Bcl-2-associated X protein
<b>ROS</b>	reactive oxygen species
<b>TERT</b>	telomerase reverse transcriptase
<b>ALT</b>	alternative lengthening of telomeres
<b>ATRX</b>	chromatin-remodeling protein
<b>DAXX</b>	death-domain associated protein
<b>PI3K</b>	phosphoinositide 3-kinase
<b>AKT</b>	protein kinase B
<b>RAS</b>	rat sarcoma virus
<b>MAPK</b>	mitogen-activated protein kinase
<b>CDKN2A/p16<sup>(INK4A)</sup></b>	cyclin-dependent kinase inhibitor 2 A
<b>TP53</b>	tumor protein 53
<b>RB1</b>	retinoblastoma 1
<b>CDKN1A/p21<sup>(CIP1)</sup></b>	cyclin-dependent kinase inhibitor 1 A
<b>Wnt (Wg + int)</b>	Wingless-related integration site
<b>CDK</b>	cyclin-dependent kinase
<b>EZH2</b>	enhancer of zeste homolog 2
<b>NK cells</b>	natural killer cells
<b>IL</b>	interleukin
<b>TAM</b>	tumor-associated macrophage
<b>ICB</b>	immune checkpoint blockade
<b>PD-L1</b>	reprogrammed death-ligand 1
<b>HLA-E</b>	human leukocyte antigen-E
<b>CAR</b>	chimeric antigen receptor
<b>uPAR</b>	urokinase-type plasminogen activator receptor
<b>EMT</b>	epithelial-to-mesenchymal transition
<b>NF- κB</b>	nuclear factor kappa-light-chain-enhancer of activated B cells
<b>C/EBPβ</b>	CCAAT/enhancer-binding protein β
<b>p38/MAPK</b>	p38 mitogen activated protein kinase
<b>mTOR</b>	mammalian target of rapamycin
<b>CCL2</b>	chemokine (C-C motif) ligand 2
<b>DDR</b>	DNA-damage response
<b>MMP</b>	matrix metalloproteinase
<b>MDCS</b>	myeloid-derived suppressor cell
<b>VEGF</b>	vascular endothelial growth factor
<b>CSC</b>	cancer stem cell
<b>IHC</b>	immunohistochemistry
<b>PET-CT</b>	positron emission tomography-computed tomography
<b><sup>18</sup>F-β-gal</b>	<sup>18</sup> F-labelled β-galactosidase tracer
<b>SA- β-gal</b>	senescence-associated beta-galactosidase
<b>Par-4</b>	prostate apoptosis response-4
<b>PROTAC</b>	proteolysis targeting chimeras
<b>BBB</b>	blood-brain barrier

# 1. INTRODUCTION

Senescence is normally a stable and irreversible cell cycle arrest, in which the cells do not proliferate in response to mitogenic signals, although they remain viable and metabolically active (Campisi and d'Adda di Fagagna 2007). These cells secrete chemicals such as cytokines, chemokines, growth factors, proteases and extracellular matrix (ECM) components, and this is called senescence-associated secretory phenotype (SASP) (Chojak et al. 2023; Salam et al. 2023). In cancer, cellular senescence can have either an anti-tumorigenic or pro-tumorigenic role (Chojak et al. 2023). Intrinsically, senescence prevents uncontrolled cell proliferation, a typical feature of cancer, thus playing an anti-tumorigenic role (Riviere-Cazaux et al. 2023). However, in cancer, the key molecular pathways that maintain cell cycle arrest are often disturbed through genetic or epigenetic modifications, allowing these senescent cells to re-enter the cell cycle, for example, after treatment completion (Carreno et al. 2021). In many cases, this leads to tumor recurrence (Chojak et al. 2023). In addition to senescence, treatment failures can be caused by tumor invasiveness, immunosuppressive microenvironment, and intratumoral heterogeneity, especially in the case of diffuse gliomas (Salam et al. 2023). These brain tumors infiltrate brain tissue and cannot be completely removed surgically. Conventional treatment options include partial surgical resection followed by radiotherapy and chemotherapy, often with Temozolomide, which are noted to induce senescence in cancer cells and surrounding cells in the tumor microenvironment. (Chojak et al. 2023) High-grade gliomas, such as glioblastoma, typically recur within the treatment field more aggressively than the original tumor. In addition, there are currently no therapies to save patients who have failed conventional treatments. (Riviere-Cazaux et al. 2023) Therefore, new therapies are urgently needed, and lately so-called "one-two punch" treatment method has been investigated. In this approach, senescence is first induced in glioma cells using known senescence-inducing factors, such as Temozolomide or radiotherapy. After that, these senescent cells are targeted with senolytics that selectively eliminate them. (Chojak et al. 2023) However, more research is needed to understand the role of senescence in gliomas, to find optimal treatment and administration strategies, and to identify reliable markers for detecting senescent cells and monitoring the effectiveness of senolytics or other senotherapeutic products.

## 2. DIFFUSE GLIOMAS

Gliomas are the most common malignant primary brain tumors (Chojak et al. 2023). Most gliomas are diffuse, meaning that the tumor infiltrates brain tissue, making complete surgical resection impossible (Chojak et al. 2023; Leppä et al. 2023). The incidence increases even in people under 20 years old but is the greatest in the elderly (Leppä et al. 2023). The prognosis for gliomas is poor (Chojak et al. 2023), and only 30% of patients are alive five years after the initial diagnosis (Leppä et al. 2023). Conventional therapies used for gliomas include partial surgical resection followed by ionizing radiation and chemotherapy with Temozolomide (Chojak et al. 2023), which aim to induce DNA damage in cancer cells (Riviere-Cazaux et al. 2023).

The World Health Organization (WHO) has updated the classification of central nervous system tumors by using molecular markers and histology for diagnostic classification and prognostication within gliomas (Melhem et al. 2022). Diffuse gliomas in adults include astrocytoma (grade 2-4), oligodendroglioma (grade 2-3), and glioblastoma (grade 4) (Leppä et al. 2023). Glioblastoma is currently referred to as the most aggressive form of isocitrate dehydrogenase (IDH)-wild type diffuse adult-type astrocytoma (Melhem et al. 2022). Overall, glioblastoma is the most common and aggressive malignant brain tumor in adults, with a median survival of only 15-21 months. (Chojak et al. 2023; Riviere-Cazaux et al. 2023) Therefore, there is an urgent need to develop more effective therapies that do not cause excessive detrimental side effects.

In glioblastoma, the O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) promoter methylation status is used as a prognostic marker (Melhem et al. 2022). Generally, prognosis is always individual, but young age, low grade of malignancy, 1p and 19q chromosome deletions in oligodendrogliomas, and IDH-mutation in diffuse gliomas can be considered favorable predictors (Leppä et al. 2023). Thus, IDH-mutant gliomas are usually less aggressive and easier to treat than IDH-wild type gliomas.

## 2.1 TREATMENT RESISTANCE

Gliomas, especially glioblastomas, are highly resistant to treatments and relapse easily (Chojak et al. 2023). There are several mechanisms by which gliomas can develop and maintain treatment resistance, some of which are common to many other cancer types as well. But especially in case of diffuse gliomas, treatment resistance can be caused by tumor invasiveness, heterogeneity, and an immunosuppressive microenvironment, for example. (Salam et al. 2023)

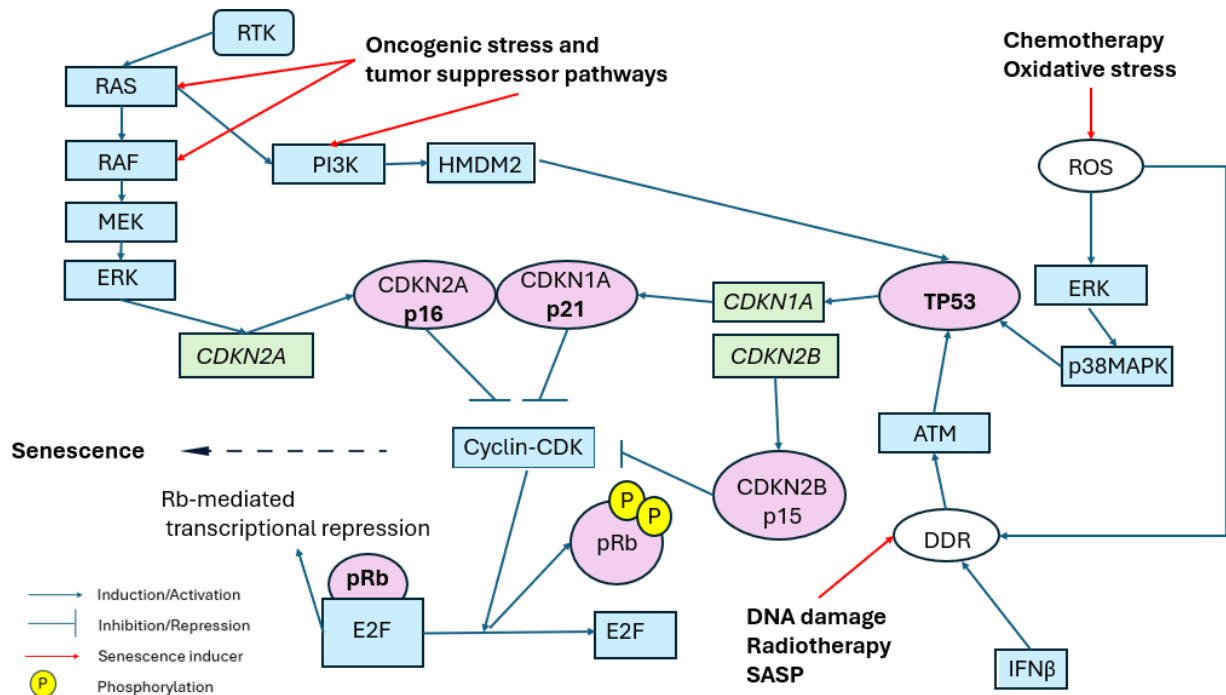
In addition to the above-mentioned mechanisms, glioma cells can acquire treatment resistance via cellular senescence (Riviere-Cazaux et al. 2023). It has been known for a while that cellular senescence inhibits tumor growth by halting the cell cycle, but lately, there has been a lot of discussion about the role of cellular senescence as a treatment resistance mechanism in cancer (Chojak et al. 2023). When DNA damage is induced in glioma cells using chemoradiation, for example, the cells can upregulate cell cycle regulatory factors such as p16, p53 and p21, and promote either DNA repair, apoptosis or senescence. Senescence allows glioma cells to escape therapy-induced apoptosis. (Riviere-Cazaux et al. 2023) This is because senescent cells are resistant to apoptotic stimuli and can upregulate pro-survival pathways by activating anti-apoptotic proteins such as B cell lymphoma 2 (Bcl-2) protein family, or by epigenetically repressing pro-apoptotic proteins such as Bcl-2-associated protein X (BAX) (Wang et al. 2022).

Eventually, the latent population of cancer cells can escape senescence after treatment completion and re-enter the cell cycle. Senescent tumor cells can also provoke the growth of other non-senescent cells in the tumor microenvironment through senescence-associated secretory phenotype (SASP) factors (Riviere-Cazaux et al. 2023). SASP factors can promote the immune clearance of senescent tumor cells, but they can also directly induce tumor growth or contribute to immune suppression that allows tumor progression (Salam et al. 2023). All these factors can contribute to tumor recurrence after treatment completion. Since diffuse gliomas have many different known and likely unknown mechanisms for acquiring treatment resistance, it is important to study diffuse gliomas in general to better target and develop treatments.

### 3. CELLULAR SENESCENCE

Cellular senescence is typically considered as irreversible and stable cell cycle arrest in either the G1 or G2 phase, in which the cells do not proliferate in response to mitogenic signals (Campisi and d'Adda di Fagagna 2007; Carreno et al. 2021; Chojak et al. 2023). This distinguishes senescence from quiescence, where normal cells can re-enter the cell cycle in response to mitogenic signals (Campisi and d'Adda di Fagagna 2007; Carreno et al. 2021). Senescent cells are flat, enlarged, and resistant to signals that induce cellular growth, proliferation, and apoptosis (Chojak et al. 2023). Senescence plays an important role in development, tissue regeneration, cell plasticity and reprogramming, aging, and age-related pathologies (Carreno et al. 2021; Chojak et al. 2023).

Senescence can be initiated by various intrinsic and extrinsic factors (Chojak et al. 2023), such as DNA damage, oncogene activation, elevated reactive oxygen species (ROS), and therapeutic agents (Salam et al. 2023), which can also activate apoptosis or other stress-induced programs (Chojak et al. 2023). Several pathways that regulate cellular senescence are presented in Figure 1. Through these pathways, stress-inducing factors often lead to the activation of tumor suppressor pathways, such as p53 and pRb, where CDK inhibitors, such as CDKN1A/p21<sup>(CIP1)</sup> and CDKN2A/p16<sup>(INK4A)</sup> work as mediators. This ultimately contributes to the senescent state of the cell. (Carreno et al. 2021; Martínez-Zamudio et al. 2017; Salam et al. 2023)



**Figure 1. Cellular senescence is regulated via many different mechanisms.** Senescence inducers often activate tumor suppressor pathways p53 and/or pRb. Senescence is mediated by CDK inhibitors, such as CDKN2A/p16<sup>(INK4A)</sup> and CDKN1A/p21<sup>(CIP1)</sup>. These mechanisms eventually induce cellular senescence. The activity of pRb is regulated by CDK-mediated phosphorylation. In senescence, pRb is active and in its hypo-phosphorylated state, which is maintained by CDK inhibitors. Figure modified from source (Martinez-Zamudio et al. 2017)

The colors of the different boxes/triangles present the following: green → genes, blue → pathways resulting in the activation of tumor suppressors, pink → tumor suppressor pathways (bold → main pathways), white → responses. The abbreviations stand for RTK = receptor tyrosine kinase, RAS/RAF/MEK/ERK = MAPK (mitogen-activated protein kinase) cascade, PI3K = phosphoinositide 3-kinase, HMDM2 = human ortholog of mouse-d-minute 2, CDKN2A (p16)/CDKN1A (p21)/CDKN2B (p15) = cyclin-dependent kinase inhibitors, E2F = group of genes that encode transcription factors, ATM = ATM Serine/Threonine kinase, IFNβ = interferon-β, DDR = DNA damage response, ROS = reactive oxygen species, ERK = extracellular signal-regulated kinase, p38MAPK = p38 mitogen activated protein kinase, TP53 = tumor suppressor 53, pRb = retinoblastoma protein, CDK = cyclin dependent kinase

Replicative senescence is caused by telomere shortening that occurs after each cell cycle, as telomeres cannot be fully replicated, imposing a limit on the number of cell divisions in normal cells, known as Hayflick's limit (Campisi and d'Adda di Fagagna 2007). Gliomas can evade replicative senescence mainly through two mechanisms. Glioma cells often have a mutation in the promoter region of the telomerase reverse transcriptase (TERT) gene, allowing the gene's overexpression. As a result, glioma cells can maintain the length of telomeres and thus divide indefinitely. The second mechanism is alternative lengthening of telomeres (ALT), which depends on homologous recombination of telomeric DNA sequences. Usually, the mechanism of ALT is caused by a loss-of-function mutation in the chromatin-remodeling protein ATRX, which normally reduces ALT activity, or a reduction in the amount of Death-Domain Associated Protein (DAXX). (Chojak et al.

2023) Through these mechanisms, glioma cells can achieve replicative immortality (Ordóñez-Rubiano et al. 2024).

Oncogene-induced senescence is caused by oncogenic stress (Chojak et al. 2023). In this case, oncogenes are activated and/or tumor suppressor genes are inactivated (Chojak et al. 2023; Ordóñez-Rubiano et al. 2024). This oncogenic signaling activates phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and rat sarcoma virus (RAS)/mitogen-activated protein kinase (MAPK) pathways (Chojak et al. 2023). Gliomas can also evade oncogene-induced senescence by acquiring mutations in genes that induce senescence inactivation, such as *cyclin-dependent kinase inhibitor 2A (CDKN2A)*, *tumor protein p53 (TP53)* and *retinoblastoma 1 (RB1)* (Chojak et al. 2023; Ordóñez-Rubiano et al. 2024).

Therapy-induced senescence is caused by genotoxic stress, such as ionizing radiation or chemotherapy with Temozolomide (Chojak et al. 2023). Temozolomide methylates guanine-rich areas in DNA, producing O6-methylguanine, which mainly induces senescence but can also induce apoptosis in targeted cells. Additionally, radiation can cause dose-dependent responses: higher dosages induce apoptosis, whereas lower dosages result in double-strand breaks in DNA, leading to p21 protein-induced senescence. However, there are several mechanisms by which gliomas can evade therapy-induced senescence. (Ordóñez-Rubiano et al. 2024) First, gliomas can activate the Wnt-signaling pathway, resulting in stabilization and nuclear translocation of  $\beta$ -catenin, which in turn induces the expression of genes needed for cell proliferation. In addition, gliomas can express surviving, which acts as an inhibitor of apoptosis and a downstream effector of cyclin-dependent kinase 1 (Cdk1), needed for repairing double-strand breaks by homologous recombination. This is one mechanism to inhibit apoptosis after chemotherapy. Also, cyclin-dependent kinase (CDK) activation of enhancer of zeste homolog 2 (EZH2) and altered glutamine metabolism are crucial for therapy-induced senescence evasion. (Chojak et al. 2023; Ordóñez-Rubiano et al. 2024)

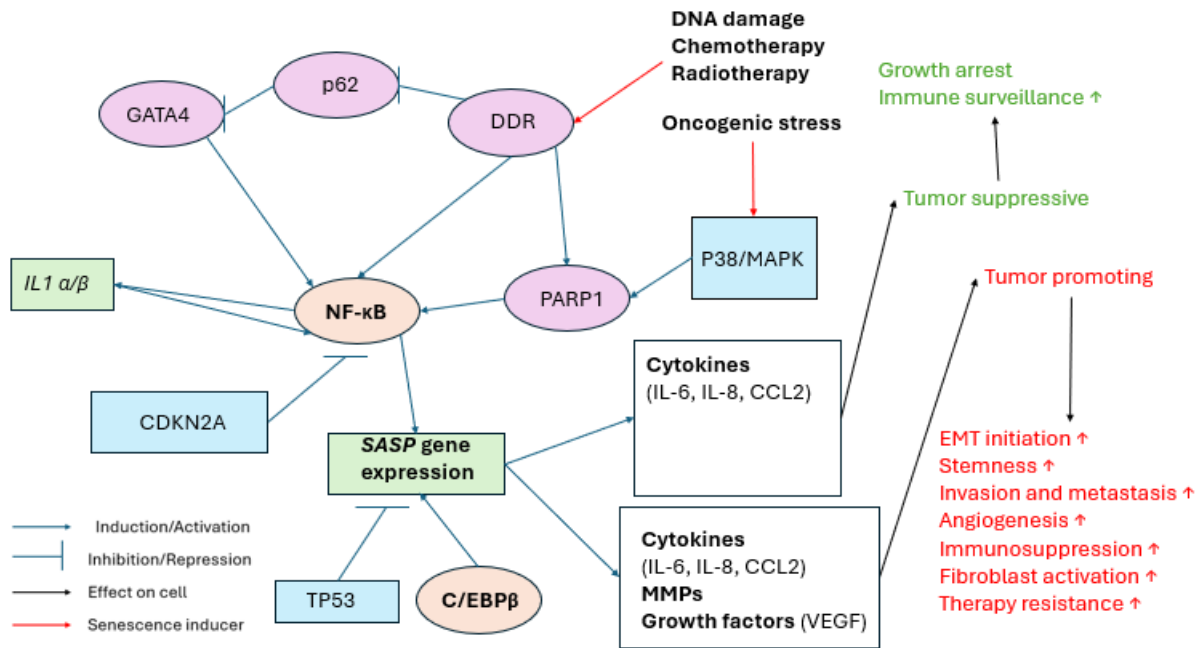
### **3.1 SENESCENCE ASSOCIATED SECRETORY PHENOTYPE (SASP)**

Senescent cells secrete chemicals such as cytokines, chemokines, growth factors, ECM components, and proteases (Chojak et al. 2023; Salam et al. 2023). This is called the senescence-associated secretory phenotype (SASP). These factors can promote invasion, metastasis formation, angiogenesis, ECM modulation, and epithelial-to-mesenchymal transition (EMT), all of which are important features of cancer growth (Chambers et al. 2021; Salam et al. 2023). They can eventually lead to therapy resistance and immunosuppression. SASP can also aid chemotherapy delivery and thus impede tumor progression. However, the cell type, tissue of origin, and primary factor that

induces senescence mostly determine how SASP influences tumor development and progression. (Chambers et al. 2021)

There are several signaling pathways, such as the mammalian target of rapamycin (mTOR), p38 mitogen activated protein kinase (p38MAPK), nuclear factor- $\kappa$ B (NF- $\kappa$ B), and CCAAT/enhancer-binding protein  $\beta$  (C/EBP $\beta$ ), which the SASP gene expression is dependent on (Carreno et al. 2021). According to Martínez-Zamudio et al. (2017) there are many regions in SASP genes in which the inflammatory transcription factors NF- $\kappa$ B and C/EBP $\beta$  can bind. These factors are induced in various ways, as presented in Figure 2. The secreted SASP factors can have either a tumor-suppressive or tumor-promoting role (Chambers et al. 2021; Wang et al. 2022), as presented in Figure 2. The effects of SASP factors are, for example, dependent on the tissue type, immune microenvironment, and the senescence inducer. Cytokines such as interleukins 6 and 8 (IL-6 and IL-8) and chemokine (C-C motif) ligand 2 (CCL2) can recruit natural killer (NK) cells and T cells, which boost immune surveillance. The autocrine role of IL-6 and IL-8 cause an increase in ROS and contributes to a sustained DNA damage response (DDR). These interleukins can also work in a paracrine manner by causing surrounding cancer cells to become senescent. (Wang et al. 2022)

However, senescence can also be induced in surrounding immune cells, causing negative effects on immune surveillance. For example, IL-6 secreted by senescent cells or released by matrix metalloproteinases (MMPs) from ECM can recruit myeloid-derived suppressor cells (MDSCs), resulting in an immunosuppressive microenvironment (Wang et al. 2022). According to Ji et al. (2024), in addition to IL-6, both IL8 and CCL2 can also recruit immunosuppressive cells and eventually induce chronic inflammation. This clearly highlights that even the same SASP of senescent cancer cells can initially have a tumor-suppressive role but a tumor-promoting role in the long term. Furthermore, many growth factors can have tumor-promoting roles by directly inducing the growth of the tumor or by EMT, which leads to metastasis formation. Moreover, vascular endothelial growth factor (VEGF) can stimulate angiogenesis, which also contributes to metastasis. (Wang et al. 2022) In addition, it has been noted that senescence and the SASP induced by chemotherapy can lead to expansion of cancer stem cells (CSCs), which usually contribute to cancer relapse (Chambers et al. 2021).



**Figure 2. Regulation of the inflammatory SASP and its effects on tumor progression.** Inflammatory transcription factors NF-κB and C/EBPβ induce SASP gene expression via different ways. Senescent cells secrete these factors, such as cytokines IL-6, IL-8 and CCL2, which can have either tumor suppressive or promoting roles. Figure modified from source (Martínez-Zamudio et al. 2017)

The colors of the different boxes/triangles present the following: green → genes, blue → pathways/proteins, pink → pathways activated through DDR, orange → inflammatory transcription factors. The abbreviations stand for NF-κB = nuclear factor-κB, DDR = DNA damage response, PARP1 = poly (ADP-ribose) polymerase 1, p62 = sequestosome-1 (ubiquitin-binding protein p62), IL-α/β = interleukin α/β, CDKN2A = cyclin-dependent kinase inhibitor 2A, TP53 = tumor suppressor 53, C/EBPβ = CCAAT/enhancer-binding protein β, GATA4 = GATA binding protein 4, p38/MAPK = p38 mitogen activated protein kinase, SASP = senescence associated secretory phenotype, MMPs = matrix metalloproteinases, CCL2 = chemokine (C-C motif) ligand 2, VEGF = vascular endothelial growth factor, EMT = epithelial-to-mesenchymal transition

### 3.2 IMMUNE CELL SENEESCENCE IN GLIOMAS

Immunosenescence can be divided into central, involving primary lymphoid organs such as bone marrow and thymus, and peripheral, involving secondary lymphoid organs such as lymph nodes and spleen. The main cell types affected by immunosenescence are hematopoietic stem cells, monocytes, macrophages, NK cells, neutrophils, T cells, and B cells. The senescence of these cells often leads to decreased infection resistance, lower vaccine effectiveness, chronic inflammation, elevated risk of autoimmune diseases, and increased risk of cancer. (Yang et al. 2025) In the context of diffuse glioma, certain immune cells can infiltrate the brain tumor microenvironment. Glioma-associated microglia and macrophages constitute a major compartment of this microenvironment. Brain macrophages can be divided into microglia in the brain parenchyma, monocyte-derived macrophages infiltrating the brain, and border-associated macrophages in the meningeal-choroid plexus-perivascular space. (Li et al. 2024)

SASP can lead to either anti-tumor or pro-tumor immunity depending on the context (Chibaya et al. 2022). Less inflammatory SASP can enhance senescent cell clearing by recruiting immune cells such as NK cells, macrophages, and cytotoxic T cells (Chambers et al. 2021). Highly pro-inflammatory SASP produced by senescent cells in the tumor microenvironment can promote the secretion of pro-tumorigenic cytokines such as IL-1a and IL-6 (Chambers et al. 2021), and induce chronic inflammation by recruiting immunosuppressive cells (Ji et al. 2024) such as MDSCs, tumor-associated macrophages (TAMs), and regulatory T cells.

In gliomas, brain macrophages are the main cells affected by SASP. Brain macrophage senescence is often detrimental because it results in persistent cell cycle arrest and can cause chronic low-grade inflammation. This leads to immune system dysfunction that can eventually contribute to glioma initiation and development. (Li et al. 2024) Chronic inflammation can also lead to treatment failures and tumor recurrence (Yang et al. 2025). Therefore, it is believed that targeting not only senescent cells but also senescent immune cells, especially macrophages, could be a potential strategy for glioma treatment (Li et al. 2024; Yang et al. 2025).

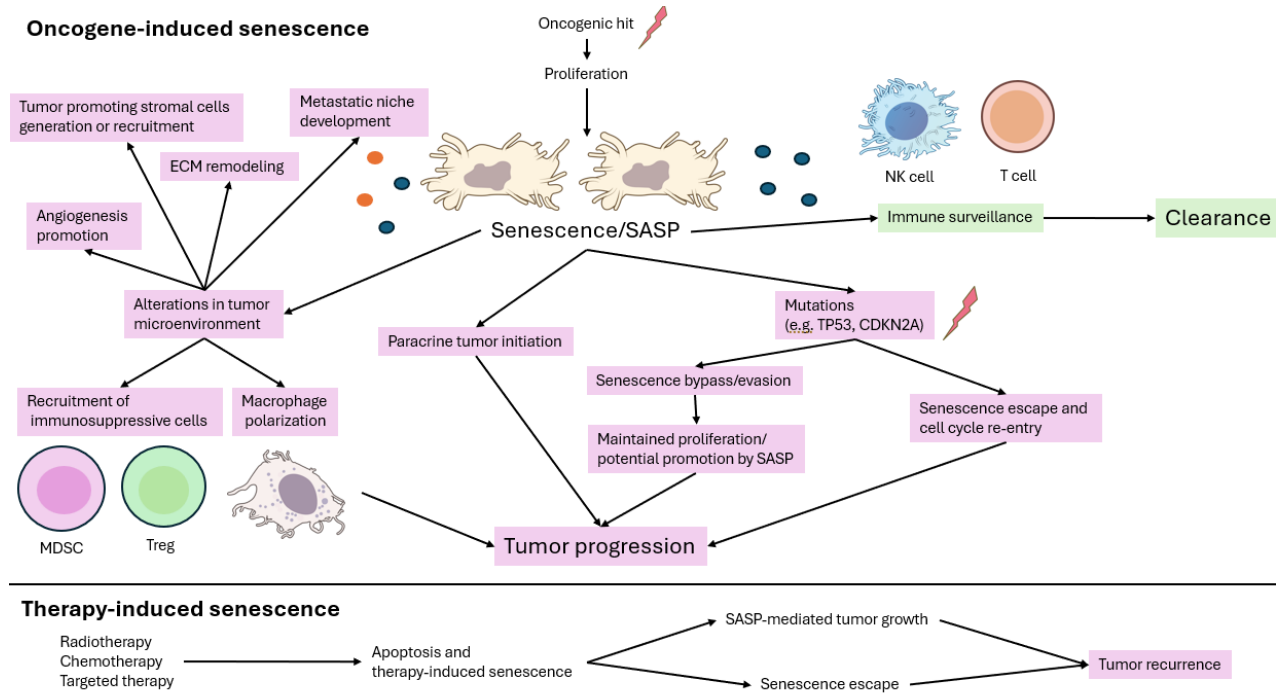
It has been noted that senescence could enhance the efficiency of immunotherapies through tumor microenvironment remodeling mediated by SASP. Therefore, senescent tumor cells could be leveraged for immunotherapy. Suitable immunotherapy methods include immune checkpoint blockade (ICB) therapies to block programmed cell death ligand 1 (PD-L1) and human leukocyte antigen E (HLA-E), chimeric antigen receptor (CAR)-T cells, and antibodies targeting senescence-related surface proteins or neutralizing antibodies targeting immunosuppressive SASP factors and their receptors. (Chibaya et al. 2022) The surface proteins of senescent cells can be used to design and engineer antibody and chimeric antigen receptor (CAR) approaches. One potential target for these is urokinase-type plasminogen activator receptor (uPAR) (Chambers et al. 2021; Chibaya et al. 2022). According to Amor et al. (2020) CAR-T cells targeting uPAR exhibit increased survival and decreased associated toxicities in mice lung adenocarcinomas. These results suggest that senolytic CAR-T cells could be used as a therapeutic approach for other senescence-associated diseases as well (Amor et al. 2020).

### 3.3 DUAL ROLE OF CANCER CELL SENESCENCE

As presented in Figure 3, senescence and SASP have dual roles in tumorigenesis. Intrinsically, senescence has an anti-tumorigenic effect by preventing uncontrolled cell proliferation and the accumulation of deleterious genetic mutations (Chambers et al. 2021; Riviere-Cazaux et al. 2023). Additionally, senescent cells often activate SASP and attract immune cells that contribute to enhanced immune surveillance and clearance of senescent cells. Because of these effects, many anti-cancer therapies aim to induce senescence in cancer cells. (Carreno et al. 2021)

Unfortunately, these therapies also generate significant amounts of senescent cells in the tumor microenvironment (Carreno et al. 2021; Chojak et al. 2023). Chambers et al. (2021) states that, since many cancer therapies are administered systematically, also normal cells within the whole area of influence become senescent in response to the cytotoxic agent. The accumulation of senescent cells is not ideal because they can drive local and systemic inflammation through SASP (Chibaya et al. 2022). Additionally, SASP factors secreted by senescent cells can contribute to the transformation of normal cells in a paracrine manner, eventually leading to tumor initiation and growth (Carreno et al. 2021). Furthermore, the inflammation caused by senescent cells can lead to secondary diseases such as fibrosis-driven disorders, cardiothoracic diseases, and neurodegenerative diseases (Chambers et al. 2021).

In addition to the generation of new senescent cells in response to conventional cancer therapies, oncogene-induced and therapy-induced senescence can also have several other pro-tumorigenic effects. Accumulation of genetic and epigenetic changes in cancer can disrupt key molecular pathways that maintain cell cycle arrest, such as p53/p21<sup>(CIP1)</sup> and/or p16<sup>(INK4A)</sup>/pRb, allowing senescent cells to re-enter the cell cycle under certain conditions (Carreno et al. 2021; Chojak et al. 2023). This enables senescent cancer cells to escape senescence, for example, after treatment completion, and eventually contributes to cancer relapse. Additionally, these mutations can allow the cells to evade senescence by preventing the establishment of a senescent response. (Carreno et al. 2021)



**Figure 3. The roles of senescence and SASP in tumorigenesis.** Upper part of the figure: Oncogene-induced senescence can lead to immune surveillance through recruitment of immune cells (e.g. NK cells and T cells) that eventually lead to clearance and suppression of tumorigenesis. Oncogene-induced senescence can also lead to tumor progression through senescence escape or bypass/evasion, via paracrine tumor initiation or via alterations in the tumor microenvironment and recruitment of immunosuppressive cells (e.g. MDSCs and Treg cells) and polarization of macrophages. Lower part of the figure: Therapy-induced senescence. Radiotherapy, chemotherapy and targeted therapy can induce apoptosis or senescence in cells in the tumor bed. Some cells remain unaffected. Senescent cells can contribute to tumor recurrence either through SASP-mediated tumor growth by inducing proliferation of the unaffected cancer cells or by senescence escape or bypass/evasion. Figure made by using some pictures from NIH Bioart Source (<https://bio-art.niaid.nih.gov/>; 22.4.2025)

The colors of different boxes present the following: green boxes = tumor-suppressive role, pink boxes = tumor-promoting role. The abbreviations stand for NK cell = natural killer cell, MDSC = myeloid-derived suppressor cell, Treg = regulatory T cell, SASP = senescence associated secretory phenotype, ECM = extracellular matrix, TP53 = tumor suppressor p53, CDKN2A = cyclin-dependent kinase inhibitor 2A

### 3.4 MARKERS FOR SENESCENT PHENOTYPE

It is important to identify senescent cells within the tumor and the tumor microenvironment both to facilitate research and to assess treatment responses. However, it is crucial to be aware that not all senescent cells are the same, as they may have different senescence inducers, originate from different tissues and cell types, and express different markers (de Magalhães 2024). Currently, it is not known what exact differences exist between senescent cancer and normal cells. Thus, further studies are required to distinguish between healthy and pathological senescent cells (de Magalhães 2024).

Because it is known that senescent cells have increased lysosomal content, the most widely used senescence biomarker is lysosomal senescence-associated beta-galactosidase (SA- $\beta$ -gal) (Campisi and d'Adda di Fagagna 2007; Chojak et al. 2023; Wang et al. 2022). In addition, pathways involved in senescence, such as p16 and p21, are also used as senescence markers. Several SASP-targeting markers are also defined, such as NF- $\kappa$ B and C/EBP $\beta$ , although it is known that certain types of senescence do not even produce SASP. The most common problems with these markers are that they are not found in every senescent cell and can be present in other cellular states and cells as well, especially in macrophages and quiescent cells. (Chibaya et al. 2022) Chibaya et al. (2022) states this is why there is an urgent need to find specific and reproducible markers that identify cellular senescence. Because the range of possible senescence markers is so wide, it is important to find the most relevant ones that specifically target the senescent phenotype. Some potential senescence markers are listed in Table 1.

However, it could be easier to identify senescent cells by using different methods rather than single-marker-based approaches to identify senescent cancer and other cells. One mechanism is to use multiplex immunohistochemistry (IHC) that enables the detection of multiple biomarker antigens on the cancer tissue (Harms et al. 2023). Senescent immune or cancer cells can be found by using markers that stain immune or cancer cells and the senescent phenotype simultaneously. For example, Salam et al. (2023) identified senescent cells from patient and mouse gliomas by using marker that targets the senescent phenotype coupled with IHC. Moreover, according to Wang et al. (2022), one potential way to detect senescent cells within a tumor is positron emission tomography-computed tomography (PET-CT) scan that uses an  $^{18}\text{F}$ -labelled  $\beta$ -galactosidase tracer ( $^{18}\text{F}$ - $\beta$ -gal). Another way is to find senescence-associated proteins or metabolites from blood and thus measure senescence burden through liquid biopsy (Wang et al. 2022).

**Table 1** Hallmarks of senescence and senescence markers, their limitations and biological responses. The most relevant markers are marked in bold. Table modified from source Carreno et al. (2021). Some of these markers mentioned in source Chibaya et al. (2022) as well.

Senescence hallmarks	Markers	Limitations	Biological responses
<b>Cell cycle withdrawal</b>	<b>Ki67 negativity</b>	also in quiescence	irreversible cell-cycle arrest
	<b>p21<sup>Cip1</sup> positivity</b>	also in quiescence	CDK2 inhibitor p21 accumulation
	<b>CDKN1A upregulation</b>		
	<b>p16<sup>INK4a</sup> positivity</b>	express by non-senescent cells (macrophages), not expressed by all senescent cells	CDK 4/6 inhibitor p16 accumulation
	<b>CDKN2A upregulation</b>		
	P15 <sup>INK4b</sup> positivity		other cyclin inhibitors accumulation
	CDKN2B upregulation		
	<b>activation of p53</b>	not specific to senescent state	gene expression regulation
	Persistent <b>activation of Rb family proteins</b>	also in quiescence	stability of the senescent state
	heterochromatinization of E2F target genes		gene silencing
<b>Macromolecular damage</b>	telomere shortening		DNA damage
	expression of PARP-1		
	expression of $\gamma$ -H2AX		
<b>Secretory phenotype (SASP)</b>	<b>NF-<math>\kappa</math>B, C/EBP<math>\beta</math>, GATA4, mTOR and p38MAPK</b> signaling pathways	high variability: cell type, inducer stimulus, and cell-to-cell variability	activation of transcription factors
	<b>IL6, IL7, IL1, IL1B, IL13, IL15, TGF<math>\beta</math>, etc.</b>		pro-inflammatory cytokines release
	<b>IL8</b> etc.		chemokines production
	EGF, HGF, FGF, <b>VEGF</b> , IGF, CXCL12, etc.		growth modulators, angiogenic factors
	<b>MMPs, TIMPs, etc.</b>		proteases, matrix metalloproteinases
	ICAM, laminin, collagens, fibronectin, <b>uPAR</b> , etc.		secretion of other factors
<b>Deregulated metabolism</b>	<b>SA-<math>\beta</math>-gal activity</b>	not in all senescent cells	lysosomes increase in number and size
<b>Senescence-associated epigenetics</b>	Histone modifications (H6K16ac, H3.3, H4K20me3, H3K9me3)		global increase in chromatin accessibility
	senescence-associated heterochromatin foci (SAHFs)		
	<b>Lamin B1 loss</b> and reduced nuclear integrity		
	upregulation of specific miRNAs		change in miRNAs expression
<b>Resistance to apoptosis</b>	increased expression of BCL-2 family members		anti-apoptotic protein upregulation <sup>1</sup>

<sup>1</sup> Ki67, nuclear protein expressed during active cell cycle; p21<sup>CIP1</sup>/CDKN1A, cyclin-dependent kinase inhibitor; p16<sup>INK4a</sup>/CDKN2A, cyclin-dependent kinase inhibitor; p15<sup>INK4b</sup>/CDKN2B; cyclin-dependent kinase inhibitor; CDK, cyclin-dependent kinase; p53, tumor suppressor protein; RB, retinoblastoma protein; E2F, groups of genes that encode transcription factors; PARP-1, poly(ADP-ribose)polymerase 1;  $\gamma$ -H2AX, phosphorylation of the histone variant H2AX; NF- $\kappa$ B, nuclear factor  $\kappa$ B; C/EBP $\beta$ , CCAAT/enhancer-binding protein  $\beta$ ; GATA4, GATA binding protein 4; mTOR, mammalian target of rapamycin; p38/MAPK, p38 mitogen activated protein kinase; IL, interleukin; TGF $\beta$ , transforming growth factor beta; EGF, epidermal growth factor; HGF, hepatocyte growth factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; IGF, insulin-like growth factor; CXCL12, C-X-C motif chemokine 12; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; ICAM, cell surface glycoprotein and adhesion receptor; uPAR, urokinase-type plasminogen activator receptor; SA- $\beta$ -gal, senescence-associated beta-galactosidase; ac, acetylation; me, methylation; Hp1 $\gamma$ , heterochromatin protein 1 gamma; miRNA, micro-RNA; BCL-2, B cell lymphoma 2

## 4. DRUGS TO TARGET SENESCENCE

Cellular senescence is currently under research to determine whether it could be utilized for the treatment of diffuse gliomas. For instance, one promising therapy strategy called “One-two punch” has been investigated. In the first line therapy, senescence is induced in glioma cells by using known senescence-inducing factors, such as chemotherapy with Temozolomide or Vincristine, radiotherapy, targeted therapy with Afatinib, or different natural compounds (Chojak et al. 2023). One natural compound that has shown to induce senescence is Thymoquinone, which is present in black cumin. It suppresses glioma cell growth by inducing the expression of the tumor suppressor prostate apoptosis response-4 (Par-4), resulting in the activation of the p53/p21 pathway that eventually leads to cellular senescence. (Subburayan et al. 2018)

In the second line therapy, senolytics are used to target and specifically eliminate senescent glioma cells (Chojak et al. 2023). Senolytics are divided into chemical and genetic types, the latter of which often work through the p16 pathway that can also induce apoptosis (Salam et al. 2023). For example, a study from Salam et al. (2023) has shown that removal of p16-expressing malignant senescent cells modifies the tumor environment and improves the survival of glioblastoma-bearing mice. Several senolytics are under study, but the most common ones are anti-apoptotic Bcl2 family inhibitors, such as Navitoclax and Venetoclax (Riviere-Cazaux et al. 2023).

In addition to senolytics, several other therapeutic strategies can target senescent cells as well. Senostatics or senomorphics are compounds that inhibit paracrine signaling by targeting the SASP phenotype but do not eliminate senescent cells. Senoreverters are used to allow senolytic cells to re-enter the cell cycle. Proteolysis targeting chimeras (PROTAC) and nanocarriers can be used to enhance the efficacy of senolytic therapies. (Chojak et al. 2023) Some therapy strategies aimed at target senescence in gliomas, their mechanism of action, known advantages and disadvantages are listed in Table 2.

As mentioned before, IDH-mutant diffuse gliomas are less aggressive and respond better to anti-cancer therapies than IDH-wild type gliomas. Zhan et al. (2021) found that IDH-mutant gliomas increased cellular senescence in glioma cells in response to Temozolomide and radiation. They propose that this may be one of the reasons why IDH-mutant glioma responses better to anti-cancer therapies (Zhan et al. 2021). One other study from Li et al. (2022) states that IDH-wild type

status is associated with a higher level of immune infiltration, immune escape, and downregulation of cell senescence-related pathways, which could be the reason for poorer prognosis. According to these findings, senolytic drugs could be effective against IDH-mutant gliomas (Zhan et al. 2021). It remains a question whether senescence could be induced effectively in IDH-wild type gliomas so that senolytics could be used.

As stated by Riviere-Cazaux et al. (2023), targeting senescence could potentially remove latent tumor cells and SASP factors from the tumor microenvironment, alleviating the long-term harmful impacts of prior therapies on cognition and bone marrow suppression induced by chemotherapy. However, the optimal administration method, dosage, and biomarkers for efficacy remain under investigation (Wang et al. 2022). For example, for senolytics to work in the brain, they need to cross the blood-brain barrier (BBB) (Fletcher-Sananikone et al. 2021). Some senolytics have also been noted to have excessive drug toxicities, which need to be considered when determining the optimal dosage (Riviere-Cazaux et al. 2023).

Also, it has been noted that senescent cells are heterogeneous and dynamic, and probably evolve over time and with changes in the tissue microenvironment (de Magalhães 2024; Riviere-Cazaux et al. 2023). According to de Magalhães (2024), there may be differences between transient short-term and chronic long-term cellular senescence. It is believed that transient senescence may be beneficial, whereas chronic senescence may be detrimental in the context of cancer (de Magalhães 2024). This raises a question about optimal timing of senolytic therapies after senescence induction (Riviere-Cazaux et al, 2023). Furthermore, because the number of senescent cells is increased in the elderly, targeting senescence can also affect the structural integrity of tissue or vascular endothelial cells. This can lead to blood-tissue barrier disorder, eventually resulting in liver and peri-vascular tissue fibrosis and overall health collapse. That is why there is an urgent need for cancer-selective senolytics. (Wang et al. 2022)

Although different combined therapies could have better outcomes than conventional therapies such as chemoradiation, the cost-effectiveness of these therapies must be considered. In general, treatment efficacy should be in line with the costs. Additionally, it needs to be predicted how many patients could be helped with these therapies, because one of the most difficult aspects of developing new cancer treatments is the individuality of cancer and treatment responses.

**Table 2** Potential senescence targeting drugs for glioma treatment.

Drug type	Mechanism of action	Known advantages	Known disadvantages	References
<b>BH3 mimetics</b>	Inhibit anti-apoptotic Bcl-2 family proteins	Can promote anti-inflammatory effects.	Drug toxicities, CNS penetration and variable sensitivity.	(Riviere-Cazaux et al. 2023)
ABT-263/Navitoclax	Bcl-2, Bcl-xL and Bcl-W inhibitor	Ablate senescent astrocytes. Selective senolytic activity after radiotherapy.	Drug toxicities, such as thrombocytopenia.	(Ordóñez-Rubiano et al. 2024; Riviere-Cazaux et al. 2023)
Venetoclax	Bcl-2 inhibitor	Increases cell death <i>in vitro</i> .	Not significant effects when comparing effects on senescent and non-senescent glioma cells.	(Riviere-Cazaux et al. 2023)
PROTACs	Bcl-xL selective proteolysis targeting chimeras	Less off-target toxicities compared to ABT-263.	Relative CNS penetration and effect in gliomas unknown.	(Riviere-Cazaux et al. 2023)
<b>Flavonoids</b>				
Fisetin	Inhibits PI3K/AKT/mTOR and AMPK pathways	Reduces the number of senescent cells after Temozolomide therapy.	No senolytic impact on some cell lines.	(Riviere-Cazaux et al. 2023)
Quercetin (combined with dasatinib)	Targets SCAPs, such as tyrosine kinases, PI3K/AKT/mTOR, p53/MDM2/p21 and HIF1 $\alpha$	Reduces circulating SASP factors. Removes senescent cells in some tissues.	No essential effects on glioma cells.	(Riviere-Cazaux et al. 2023)
<b>Targeting Apoptosis family inhibitors</b> BV6	Inhibition of inhibitors of apoptosis, such as c-IAP1 and c-IAP2	In combination with Venetoclax and Temozolomide increases apoptosis.	-	(Riviere-Cazaux et al. 2023)
<b>Senomorphics</b> Tocilizumab	Target IL-6 Target downstream signaling of SASP factors	Decreases growth of parts of glioma. Decreases glioma stem cell growth.	-	(Riviere-Cazaux et al. 2023)
<b>Antimalarial</b> Artesunate	Not entirely defined	Senolytic activation in several cell lines.	-	(Riviere-Cazaux et al. 2023)
<b>Hsp90 inhibitors</b> Onalespib	Inhibitor of Hsp90	Cytotoxic impact on glioblastoma.	Cytotoxic impact is not specific to senescent cells.	(Riviere-Cazaux et al. 2023)
<b>Telomerase inhibitors</b>				
Imetelstat	Targets RNA component of telomerase	Inhibits growth of glioma cells. Increases radiosensitivity.		(Ordóñez-Rubiano et al. 2024)
Abacavir	Antiretroviral nucleoside analogue used to treat HIV/AIDS Inhibition of telomerase	Reduces cell growth and promotes differentiation in medulloblastomas.	Not known effect in glioma cells.	(Ordóñez-Rubiano et al. 2024) <sup>2</sup>

<sup>2</sup> BH3 mimetic, mimic BH3-only proteins; BCL-2, xL, -W, B cell lymphoma 2, extra-large, W; CNS, central nervous system; -ib, inhibitor; PI3K/AKT/mTOR, phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin; AMPK, AMP-activated protein kinase; SCAP, senescent cell anti-apoptotic pathway; p53/MDM2/p21, tumor suppressor p53/mouse double minute 2 homolog/cyclin-dependent kinase inhibitor p21; HIF1 $\alpha$ , hypoxia-inducible factor 1-alpha; SASP, senescence associated secretory phenotype; c-IAP, cellular inhibitor of apoptosis protein; -mab, monoclonal antibody; IL, interleukin; Hsp90; HIV/AIDS, human immunodeficiency virus/acquired immune deficiency syndrome

## 5. CONCLUSIONS

Because diffuse gliomas are the most common malignant primary brain tumors and are resistant to many currently used treatments and prone to relapse, it is important to study these tumors more deeply to find new, more effective treatment methods. Diffuse gliomas have several ways to acquire treatment resistance and to recur. Cellular senescence, which is normally defined as irreversible and stable cell cycle arrest in which the cells remain viable and secrete various chemicals, is one key mechanism. There are several types of senescence: replicative, oncogene-induced, and therapy-induced senescence, each induced by different primary factors. Gliomas can evade all types of senescence through various mechanisms, mainly due to acquired mutations, epigenetic modifications, and alterations in signaling pathways.

Moreover, senescence and its associated secretory phenotype, SASP, can have a dual role in the context of cancer. Because senescence results in cell cycle arrest, it inhibits the proliferation of cancer cells. The produced SASP factors can recruit immune cells that increase immune surveillance. However, these SASP factors secreted by senescent cancer and/or other cells can also recruit immunosuppressive cells and cause other alterations in the tumor microenvironment that eventually lead to tumor initiation or progression. Senescence escape and evasion can also contribute to tumor progression. These mechanisms can also lead to tumor recurrence after therapy-induced senescence.

It is important to study these mechanisms of senescence induction, evasion and escape because conventional treatments used for diffuse gliomas can induce senescence not only in cancer cells but also in other cells in the tumor microenvironment, such as in immune cells. It is currently under research whether senescence could be utilized for new glioma treatments. Several senescence-targeting drugs, senolytics and senostatics, are under active investigation for combined therapies. In addition, senescence has an essential role in other age-related diseases such as neurodegenerative conditions. Maybe in the future, knowledge of senescence could also be utilized for the treatment of these diseases, tissue regeneration, or slowing down aging in general.

However, there is currently an urgent need to find more reliable, specific, and reproducible markers for identifying senescent cells. Although several markers have been identified, they are usually not truly specific to the senescent state and/or are not expressed by all senescent cells. It could also be useful to have these markers for monitoring treatment responses.

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