OPINION

Pro-senescence therapy for cancer treatment

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Abstract | Abundant evidence points to a crucial physiological role for cellular senescence in combating tumorigenesis. Thus, the engagement of senescence may represent a key component for therapeutic intervention in the eradication of cancer. In this Opinion article, we focus on concepts that are relevant to a pro-senescence approach to therapy and we propose potential therapeutic strategies that aim to enhance the pro-senescence response in tumours.

In recent years, our understanding of the pathology of cancer has highlighted the relevance and role of senescence as a physiological barrier against tumour initiation and progression. Indeed, there is a large body of emerging literature that now demonstrates that the role of senescence in opposing tumour growth *in vivo* had previously been underestimated, thereby advocating the incorporation of a pro-senescence component to therapy.

The first description of 'cellular senescence' dates to 1965 when Leonard Hayflick[1] observed that cells undergo a replicative senescence in culture (TIMELINE). It is now well established that premature forms of cellular senescence can be triggered through either the activation of oncogenes (a type of senescence that is termed oncogene-induced senescence (OIS))[2] or the loss of tumour suppressor genes, including *PTEN*, *RB1*, *NF1* and *INPP4B*[3–5,104]. Our work has specifically characterized the senescence response that is induced by the loss of the tumour suppressor gene *PTEN*[3], and we have termed this PTEN loss-induced cellular senescence (PICS) (TIMELINE).

In this Opinion article we focus on the therapeutic potential of senescence for cancer therapy. Initially, we outline the unique features of the differential senescence responses that have been characterized to date and discuss important aspects that render the pro-senescence approach attractive for therapeutic exploitation. Additionally, we discuss aspects that may require optimization to fully benefit from the implementation of pro-senescence therapy. Finally, we propose several therapeutic strategies that allow for the inclusion of pro-senescence therapy as part of cancer treatment protocols.

Differential senescence responses

There has been much debate concerning the physiological relevance of senescence *in vivo*, particularly as this response was originally identified as an *in vitro* phenomenon. Ultimately, however, compelling data have emerged that demonstrate the relevance of cellular senescence *in vivo* in both mouse[3,6–8] and human[4,9–12] tumours (as reviewed in REF. 13). Nevertheless, we propose that the possibility that the antitumour role of senescence could be harnessed for cancer therapy and prevention has been completely underestimated to date. Therefore, the induction of senescence for therapeutic gain represents a novel, but unexplored, approach to promote disease eradication.

Commonly recognized characteristics of cellular senescence include morphological features (large and flat morphology), the presence of vacuoles, positive staining for the senescence-associated β-galactosidase marker (SA-β-Gal) and the engagement of key effector pathways (for example, the p53 and RB pathways[14]). However, a more in-depth analysis has revealed several distinct forms of senescence that are each characterized by unique features.

Replicative senescence. Replicative senescence results from a combination of events that includes the progressive erosion of telomeres owing to the accumulation of replication cycles[15–17] and the increased expression of cyclin-dependent kinase inhibitors (CDKIs)[18,19]. In particular, telomere erosion can result in critically short telomeres that are sensed by the cells as double-strand breaks (DSBs) in DNA[20], which in turn promotes replicative senescence (FIG. 1a). These DSBs elicit a DNA damage response (DDR) to transduce signals and to execute senescence, resulting in the formation of γ-H2AX-positive senescence-associated DNA damage foci (SDF)[20] and the activation of the ATM and ATR kinases, which signal downstream to p53 (REF. 20). In addition, both INK4A (also known as p16; a CDKI that operates upstream of RB) and the RB tumour suppressor have important roles in replicative senescence[14,21] (FIG. 1a).

Importantly, by inactivating the RNA moiety that is required for telomerase activity (*Terc*), several mouse models have addressed the role of telomerase function in cancer[22,23]. In most cases these mice clearly demonstrate the importance of telomerase for tumour development, with significantly reduced tumour formation in $Terc^{-/-}$ mice, and activation of cellular senescence with increases in the activity and expression of senescence-associated effectors (for example, p53 and p21)[24,25]. However, it should also be noted that in some contexts the abrogation of *Terc* (for example, in $Terc^{-/-}$;$Trp53^{-/-}$ mice) can alter the range of tumours, leading to an increased incidence of some tumour types owing to dramatic changes in cellular ploidy[24,26].

Oncogene-induced senescence. OIS was originally shown to be an *in vitro* response triggered by cells to prevent oncogenic transformation[27] (TIMELINE). In the case of OIS induction that is driven by $HRAS^{G12V}$ overexpression in human fibroblasts it

has been established that this response is triggered by hyperproliferation and DNA hyper-replication[10,28,29] (FIG. 1b). This in turn sparks the activation of an S phase-specific DDR[28]. Although this DDR is initiated by a mechanism that is distinct from that of replicative senescence, it shares effectors and primary pathways and also results in SDF formation[30]. Indeed, OIS fails in cells that lack ATM activity or when cells cannot sense DNA damage or transduce DDR signals to p53 (REF. 28). p53 itself represents an important effector of OIS[31,32], through transcriptional activation of target genes, including *CDKN1A* (encoding the protein p21)[27]. In OIS, the activation of p53 is driven by phosphorylation[28], with concomitant stabilization of the protein mediated by ARF induction[27] (FIG. 1b). However, ARF induction may have a more limited role for the upregulation of p53 in a human context[33]. In addition to p53, OIS also engages other senescence effectors, including INK4A (for example, through ETS2 (REF. 34) and derepression of the genomic locus through inactivation of the polycomb group complex[35]) (FIG. 1b).

Although mechanistic details regarding OIS induction have been elucidated *in vitro*, there are now several mouse models providing physiological evidence for OIS *in vivo*[6,7,36–38]. Interestingly, the senescence response that is induced through the loss of the *Rb1* tumour suppressor gene *in vivo* has also been reported to display OIS-like features that are mediated by activation of NRAS[5]. Together, these mouse models demonstrate that senescence is a primary response that is elicited to antagonize tumour progression at an early stage of tumorigenesis.

PTEN loss-induced cellular senescence. PICS represents a distinct form of senescence that is rapidly induced on *Pten* loss and that is characterized by the absence of an 'OIS-like' hyper-replication, as well as a lack of a DDR[39] (FIG. 1c). Indeed, PICS can occur in cells that are treated with aphidicolin, which blocks S phase entry and prevents DNA replication[39]. This is different from $HRAS^{G12V}$-induced OIS, in which aphidicolin treatment results in the abrogation of senescence[28]. Furthermore, the SDF formation is also not observed in PICS, and inhibition of ATM has no effect on PICS induction[39].

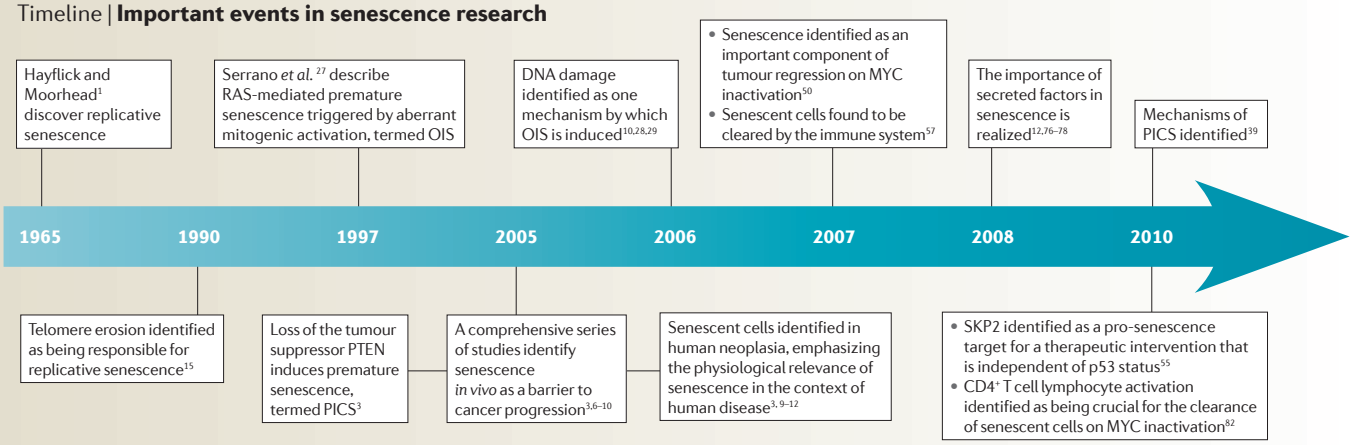
Similar to OIS, p53 has an important role in PICS. However, in this context p53 upregulation is mainly promoted by mTOR-mediated translation[39,40] (FIG. 1c). In addition, the genetic inactivation of the gene encoding ARF at the *Cdkn2a* locus does not dramatically alter p53 levels on the induction of PICS[39–41], although it results in a marked reduction of p53 in OIS[42]. Furthermore, genetic deletion of the gene encoding ARF at the *Cdkn2a* locus *in vivo* fails to prevent PICS induction in prostate tumorigenesis, demonstrating the dispensable nature of ARF for PICS[41]. We have also recently uncovered a novel role for nuclear PTEN that contributes to PICS[43]. This role for PTEN is independent of its phosphatase activity and promotes the upregulation of INK4A through the regulation of ETS2 (REF. 43). Thus, PTEN loss drives senescence from two perspectives: through p53 upregulation resulting from mTOR hyperactivation, and through INK4A upregulation resulting from disassembly of the CDH1-containing anaphase-promoting complex (APC/C (also known as the cyclosome)–CDH1) and subsequent accumulation of ETS2 (FIG. 1c).

Importantly, genetic deletion of *Pten* in the mouse prostate has shown the relevance of PICS *in vivo*[3]. Furthermore, concomitant genetic inactivation of *Trp53* with *Pten* in the prostate illustrates the importance of PICS in preventing the rapid progression of pre-malignant lesions to aggressive cancer[3] (TIMELINE). The dramatic consequence of PICS evasion *in vivo* has been further corroborated in subsequent studies[41,44]. As is the case for OIS, these models demonstrate the crucial and initial stage at which PICS blocks the transformation of early lesions to malignant prostate cancer. Although it is clear that PICS has a central role in blocking tumour progression in prostate tumorigenesis, the extent to which PICS might restrict the development of other tumours is not clear. It should be noted that although senescence has been hypothesized to have a role in the haematopoietic stem cell compartment on *Pten* loss, this has not yet been proved[45]. However, although senescence has not been reported on loss of *Pten* in other tissues (for example, the pancreas[46]), it is currently unclear whether these tissues demonstrate an intact PICS response.

From a therapeutic perspective, the induction of PICS, or a PICS-like senescence, offers several advantages over the induction of OIS.

First, on the basis of previous reports[10,28,29,47] it might be expected that, in contrast to PICS, the coupling of hyper-replication and the subsequent accrual of DNA damage in $HRAS^{G12V}$-driven OIS might result in an increased risk that cells experience genomic instability leading to secondary mutations. This can allow an escape from DDR-driven OIS and the initiation of tumorigenesis. On the contrary, PICS is induced in the absence of such a

Timeline | **Important events in senescence research**



OIS, oncogene-induced senescence; PICS, PTEN loss-induced senescence; SKP2, S phase kinase-associated protein 2.

hyperproliferative burst and a DDR. It is also activated over a much shorter time, thereby avoiding the genomic instability and the risk of acquiring secondary mutations. Nevertheless, to avoid the potentially tumorigenic consequences of engaging OIS by upstream activation, targeting downstream OIS effectors may be beneficial in uncoupling the hyper-replication and subsequent DDR from the senescence induction.

Second, the absence of a requirement for DNA replication for PICS also suggests that PICS may be induced in quiescent or growth-arrested cell types. This is highly relevant, as several models describing the pathogenesis of cancer implicate the presence of a small number of quiescent cancer-initiating cells (CICs) that contribute to the maintenance of the tumour, and fail to be targeted by current therapeutic protocols[48]. Although the induction of OIS may have the potential to reduce tumour burden in general, quiescent CICs might escape the activation of OIS, as senescence that is driven by mutant RAS suggests a dependence on hyper-replication, a characteristic that quiescent CICs lack.

Finally, as p53 activation in PICS is predominantly translational, there is the potential to boost p53 levels further using therapies that target MDM2 (for example, through the use of nutlins[49]). On the contrary, OIS induction heavily depends on ARF to eliminate MDM2 for p53 stabilization (primarily in mouse systems). Indeed, in contrast to OIS, the treatment of cells undergoing PICS with nutlins can greatly increase p53 levels[39]. Thus, PICS represents an attractive therapeutic option given the relevance of p53 induction as a barrier to tumorigenesis.

Senescence in the eradication of established tumours. Several mouse models of tumorigenesis that are driven by MYC have recently demonstrated the importance of senescence, not only for pre-malignant tumours, but also for tumour regression upon oncogene inactivation in established tumours[50]. MYC-mediated tumorigenesis represents a paradigm for the phenomenon that is known as oncogene addiction[51], whereby tumours that are driven by oncogene overexpression remain dependent on the constant expression of the oncogene itself for their maintenance and progression. In this context, constitutive overexpression of MYC allows tumours to arise that have overcome early checkpoints to result in aggressive disease[50].

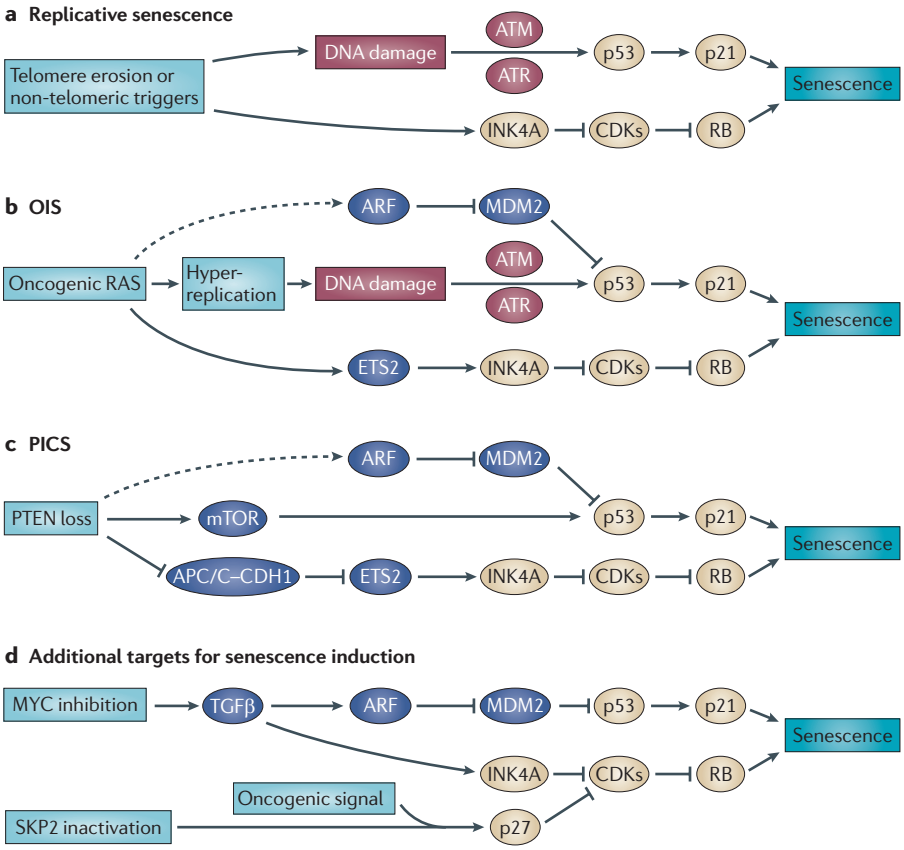


Figure 1 | **Differential senescence responses.** Several independent stimuli have the capacity to induce senescence through various common effectors. These differential stimuli can be categorized as replicative senescence, oncogene-induced senescence (OIS) and PTEN loss-induced cellular senescence (PICS). Upstream effectors are shown in dark blue, DNA damage transducers in red and downstream effectors in yellow. **a** | Replicative senescence is driven by multiple stimuli, including telomere erosion, and can result in activation of INK4A (also known as p16) expression and can trigger DNA damage pathways resulting in p53 induction. **b** | In OIS, the activation of p53 is driven by two main mechanisms. First, it is stabilized through phosphorylation by the DNA damage response (DDR) and second, by ARF-mediated stabilization. The dashed arrow is used to emphasize the fact that although ARF is an important factor for senescence induction in mouse cells, it does not seem to have such a crucial role in a human context. **c** | By contrast, p53 upregulation in PICS is mainly mediated through translational mechanisms that are controlled by mTOR. In addition, the ETS2–INK4A pathway is also required for senescence induction. Although RAS–MAPK signalling directly promotes ETS2 activity[34], PICS-mediated activation of ETS2 occurs through the deregulation of ETS2 degradation by the CDH1-containing anaphase-promoting complex (APC/C (also known as the cyclosome)–CDH1)[43]. **d** | Senescence induction can also be achieved through targeting key inhibitors of senescence. For example, MYC inactivation can result in the restoration of transforming growth factor-β (TGFβ) signalling pathways resulting in senescence, and the inhibition of S phase kinase-associated protein 2 (SKP2) in combination with additional oncogenic events (for example, RAS activation or PTEN loss) can also drive senescence. CDK, cyclin-dependent kinase.

The inactivation of MYC results in a strong induction of antitumour mechanisms, including senescence, to promote the elimination of the cancer. Importantly, cellular senescence has been identified as a specific mechanism to counteract various tumour types, including lymphoma, osteosarcoma and hepatocellular carcinoma[50]. However, in order to render tumours sensitive to senescence induction, it is necessary that key mechanistic and environmental cues are in place to allow tumour regression. These include the presence of competent p53 and INK4A and sensitivity to the transforming growth factor-β signalling pathway (FIG. 1d).

In addition to extensive studies that highlight the relevance of p53 for senescence, there is also a large body of literature that focuses on the important role of RB and the CDKIs[52,53]. Notably, to fully bypass senescence in mouse embryonic fibroblasts (MEFs) it is typically sufficient to block either p53 or RB family members,

although both pathways must be blocked in human cells[54]. However, the generation of several *in vivo* mouse models has been instrumental in disentangling the p53 components and thereby highlighting the relevance of p53-independent pathways in driving senescence[13]. Importantly, we have also shown that in an oncogenic setting (for example, the overexpression of $HRAS^{G12V}$ or the loss of *Pten*) senescence can be triggered in a p53-independent manner, whereby p27 (also known as KIP1) activation — through reduced levels of the major E3-ubiquitin ligase responsible for p27, S phase kinase-associated protein 2 (SKP2) — represents an important component of senescence induction[55] (FIG. 1d).

The models discussed above demonstrate two key advantages that are relevant to pro-senescence therapy. First, MYC inactivation shows that senescence can be induced even in established tumours. Second, the ability of SKP2 inhibition to drive senescence in the context of p53 inactivation indicates that senescence can also be achieved in a setting of p53 deficiency, which is frequently associated with many tumour types.

Considerations for therapy

The concept of pro-senescence therapy has emerged over the past few years as a novel therapeutic approach to treat cancers. However, unlike the field of apoptosis, which was embraced with great fervour in its promise to cure cancer by cell suicide[56], there has been little excitement surrounding the use of senescence as a cancer therapy strategy. This scepticism mostly arose from the prevailing dogma that senescent cells were not cleared by the immune system, but remained part of the tissue. By contrast, it is now becoming evident that cellular senescence is a robust physiological antitumour response that is engaged by tissues to counteract oncogenic insults. Not only does it appear to be one of the primary physiological actions taken to inhibit tumorigenesis, but it is also now evident that senescent cells can be cleared *in vivo* through an innate immune response[57].

A common response to existing therapies? Traditional cancer chemotherapies have involved the use of single agents or combinations of cytotoxic compounds to combat cancer[56]. This approach primarily aims to induce extensive DNA damage, thereby killing rapidly dividing tumour cells. What is now apparent, and which was overlooked in the past, is how these standard therapies often trigger a potent senescence response. For example, evidence in the literature has shown that the expected apoptotic response achieved through the use of chemotherapy and ionizing radiation treatments is also accompanied by a robust and concomitant induction of senescence[58–61]. However, the true extent to which senescence forms part of the response to such treatments will only be fully realized once we can properly follow and quantify senescence in human patients (for example, through *in vivo* imaging or molecular biomarker assays). Although chemotherapy and radiotherapy have considerably improved both the overall survival and the disease-free survival of cancer patients, perhaps in part through senescence induction, most chemotherapies also target normal rapidly proliferating cells, therefore demonstrating particularly high toxicity for organs that require active proliferation (for example, the bone marrow, the gastrointestinal tract and hair follicles).

Cancer cell-specific targeting. In an effort to develop better therapeutics, targeted therapies have emerged that can greatly reduce the toxicity that is associated with traditional therapies. This approach takes advantage of tumour-specific characteristics that are not found in normal cells. Examples of such targeted therapies include the effective treatment of patients with breakpoint cluster region (BCR)–ABL1-positive chronic myeloid leukaemia (CML) using imatinib[62]; the treatment of acute promyelocytic leukaemia (APL) with all-*trans* retinoic acid (ATRA) in combination with arsenic trioxide[63]; and the treatment of HER2 (also known as ERBB2)-positive breast cancers with the monoclonal antibody trastuzumab[64]. Although targeted therapies have been more successful in the treatment of haematological malignancies, their application to the treatment of solid tumours has been less rewarding. Notably, many therapies that alter signalling events within cells result in the activation of various in-built feedback mechanisms that exist to normally regulate these pathways, shunting signals to result in the activation of alternative signalling routes[65,66]. In this respect, a pro-senescence approach that is directed at specific modulators of senescence can also be regarded as a form of targeted therapy, and like other such approaches targets specific proteins and pathways to drive the tumour cells to irreversible growth arrest. However, as for other targeted therapies, we cannot exclude the possibility that potential feedback mechanisms may exist; however, given the apparently irreversible nature of the senescence process, the presence and relevance of such feedbacks remain to be determined.

Senescence in the context of cancer genetics. As mentioned briefly above, the genetic lesions that drive cancer imply that the use of senescence as an anticancer mechanism can occur either at an early or at a late stage of tumour development. As in the case of OIS and PICS, the genetic lesions that initiate tumorigenesis (that is, RAS activation in OIS or PTEN loss in PICS), promote senescence at an early and primary stage of tumorigenesis[3]. By contrast, established tumours, such as those observed in mouse models for MYC-driven tumours, demonstrate the importance of senescence induction as an essential component of tumour regression on MYC inactivation[50]. In addition, a targeted pro-senescence approach also incorporates the emerging concept and the power of synthetic lethality. The ability to target a specific gene, the inactivation of which alone does not affect normal cellular homeostasis but can result in a lethal outcome in the context of cells with a cancer-specific mutation, can also be applied towards pro-senescence therapy. This application can be either in the form of true synthetic lethal interactions, as is reported for oncogenic *KRAS* and selective mitotic genes[67], or in the form of a 'synthetic senescent interaction', as has been described in the recent reports for the interaction of activated *Kras* with *Cdk4* loss[68]; MYC overexpression with *Cdk2* loss[69]; and for p53 loss of function with SKP2 targeting[55].

Senescence targeting of the quiescent CICs. Finally, as highlighted above, we propose that a key and unique advantage of PICS-based pro-senescence therapy is that it may offer the potential to target the quiescent CIC population: suggesting a genuine 'quiescence to senescence' approach. Therefore, PICS may potentially inactivate both the proliferating bulk of the tumour and any non-proliferating quiescent-like cells that also contribute to tumour maintenance.

Optimizing senescence-based therapies

The use of senescence for therapy requires several elements to be in place for effective optimization and implementation, to monitor the efficacy of such treatments and to gain optimal benefit from them. Two key components that are required to achieve these objectives include an ability

to detect and quantify senescence *in vivo* and the recruitment of immune function to aid in the clearance and regression of disease.

Detection. As previously reviewed by Collado and Serrano[13], *in vivo* modelling and evidence from human tumours have demonstrated the presence of senescent cells in pre-malignant lesions, and key to the effective treatment of cancer is its early detection and suppression. Thus, the ability for an early detection of this initial antitumoural response *in vivo* may offer a unique tool that allows for early intervention, and may suggest the enhancement of senescence as a therapeutic option. The classical method for detecting senescence has been to test for SA-β-Gal activity in cells using the substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside)[70]. This type of analysis may be readily carried out on tumour biopsy samples from patients in combination with immunohistochemical (IHC) analysis for the traditional senescence effectors, although it should be noted that biopsy samples should be frozen for optimal senescence analysis[71]. These markers include the upregulation of p53, INK4A and p21, in addition to the upregulation and localization of p27. Furthermore, there have been an increasing number of novel senescence markers identified in human cell lines[7], and some of these have been successfully validated *in vivo* in mice, including the basic helix–loop–helix transcription factor BHLHE40 (also known as DEC1) and decoy receptor 2 (DCR2; also known as TNFRSF10D)[7,28,38,57,72–74].

Notably, advances in imaging technologies have enabled the development of *in vivo* imaging of tumours using novel fluorescence-based molecules. Indeed, the fluorescent galactoside conjugate DDAOG (7-hydroxy-9H-(1,3-cichloro-9,9-dimethylacridin-2-one galactosidase) produces, on β-Gal-mediated cleavage, a product that has far-red fluorescence properties that are detectable by imaging[75]. Importantly, the cleaved substrate shows a 50 nm red shift, enabling its specific detection in a background of intact probe, a highly desirable feature for *in vivo* imaging[75]. This substrate has already been shown to have the ability to detect the expression of the bacterial β-galactosidase (LacZ) reporter *in vivo*, with LacZ expression readily detected from xenograft implants of LacZ-expressing cell lines in mice (or mouse models engineered to express LacZ in the brain) using near infrared fluorescence (NIRF) imaging[75]. These data suggest that the DDAOG substrate may be a valuable *in vivo* imaging tool for senescence[75]. The development of such substrates as tools for detection will enable the detection and quantification of senescence in patient tumours to establish which tumour types contain a substantial proportion of senescent cells, and also to allow us to understand the extent to which standard therapies invoke a senescence response as part of their therapeutic effect. However, in developing such imaging tools it is important to keep in mind that factors such as cellular pH are relevant for the effective measurement of SA-β-Gal activity. Although it is known that SA-β-Gal is normally associated with the acidic lysosome component within the cell, it remains to be determined whether substrates such as DDAOG can be effectively used *in vivo* for senescence detection without further modifications. Such imaging tools may be invaluable in allowing therapeutic intervention at an early stage of tumour development, prior to the progression of tumours to a late aggressive state.

Immune response. The role of the immune system in regulating the response to therapy represents a formidable defence network that can aid in the fight against disease, and the efficient activation of the immune response may facilitate its regression. However, the ability of the immune system to clear senescent cells remains an area of debate. In some cases it is known that senescent cells are not completely cleared by the immune system, such as in the observation of increased numbers of senescent cells in the skin of ageing humans[70]. The accrual of these cells may represent a distinct disadvantage for pro-senescence therapy. It has been suggested that uncleared senescent cells may also have the potential to promote tumorigenesis[12,76–78]. For example, the senescence-messaging secretome (SMS)[79] from senescent cells may provoke tumorigenesis through the ability to promote the proliferation of neighbouring cells or through the secreted cytokines altering the immune response (for example, macrophage switching and the promotion of angiogenesis)[80,81]. However, the immune system may also have the potential to clear senescent cells. For example, in a mouse model of liver cancer development and progression, Xue *et al.*[57] demonstrated that the re-activation of p53 can result in the induction of senescence and the concomitant clearance of the senescent cells through the secretion of inflammatory cytokines and other factors that promote the infiltration of the tumour with innate immune cells (such as neutrophils, macrophages and natural killer cells), thereby contributing to tumour regression. Independent studies have also demonstrated that senescent cells can secrete the pro-inflammatory cytokines interleukin-6 (IL-6) and IL-8, and it is likely that this results in the recruitment of immune cells to sites of senescence[82]. In addition, an important role for $CD4^+$ T cells in the induction of cellular senescence and the regression of tumour burden on MYC inactivation has also recently been described, further emphasizing the relevance of the immune response in this process[77]. Thus, these data suggest that promoting immune cell function, while also engaging a pro-senescence response, may prove to be beneficial for the clearance of senescent cells and may result in the rapid reduction of the tumour burden. Although the relevance of these data for the treatment of human cancers remains to be determined, the implementation of adoptive cell transfer (ACT) therapies may prove to be a useful method to promote immune function in patients for the clearance of senescent cells. In ACT, tumour-reactive T lymphocytes are generated *ex vivo* from endogenous tumour-infiltrating lymphocytes, and are subsequently activated and expanded for reinfusion into the patient[83]. Thus, the combination and optimization of such immuno-modulatory approaches with senescence-enhancing agents should be considered when evaluating pro-senescence modalities as a tool for effective therapy.

Strategies for senescence induction

In developing therapeutic strategies for pro-senescence therapy, we should be mindful that many conventional therapies induce senescence as part of the therapeutic outcome, as discussed above. However, we propose several targeted therapeutic approaches to activate specific senescence programmes or common senescence effectors (such as p53, which contributes to both PICS and OIS) to specifically induce a senescence response for tumour inhibition. Additionally, we highlight a number of targeted therapies that are currently in development that are not considered 'pro-senescence' *per se*, but which should engage a senescence response as part of the outcome (for example, MYC inactivation or telomerase inhibition). Thus, we can

subdivide pro-senescence drugs into several categories. These include drugs that can enhance p53 activity and function; drugs with the ability to modulate the cell cycle machinery and status, as well as RB activity through targeting CDKs and CDKIs; drugs that target dominant oncogenes that are associated with oncogenic addiction; agents that enhance PICS; and telomerase inhibitors for the induction of replicative senescence (FIG. 2; TABLE 1). We believe that there is also the possibility of combining these targeted approaches with conventional therapies to improve the outcome and efficacy of both.

p53-enhancing approaches. The p53 tumour suppressor is an important effector for the therapeutic induction of senescence; therefore, its status in human tumours is a relevant determinant that dictates pro-senescence approaches to treatment. Intensive efforts have focused on understanding the role and importance of p53, the manner in which it is activated and the effect of mutation on its structure and function[84]. These studies have in turn led to the development and identification of several novel small molecules that promote and restore p53 activity (FIG. 2a). One of the first types of molecules identified, nutlins, were found to inhibit the ability of MDM2 to interact with p53 (REF. 49). This in turn results in the stabilization of p53 and promotes the normal function of the protein in eliciting its senescence response[49]. In fact, preclinical models examining a PICS-based pro-senescence therapy already indicate that the use of nutlins may dramatically enhance the p53 response observed in this model[39]. Additionally, innovative approaches to restoring normal p53 function may be particularly useful in pro-senescence therapy, especially as many tumours display loss of p53 activity through mutation of its DNA-binding domain (DBD)[85]. These DBD mutations seem to either alter the folding of the wild-type protein, so that the protein cannot interact with the DNA and activate transcription, or affect key structural contacts to DNA. The recent identification of drugs such as PRIMA-1^{MET} (also known as APR-246) and ellipticine has demonstrated that reactivation of mutant p53 is also possible[86,87]. These drugs can interact with mutant p53, resulting in structural changes and a reversion to wild-type activity, restoring normal transcriptional activation of p53 targets, including *CDKN1A*[86] (FIG. 2a).

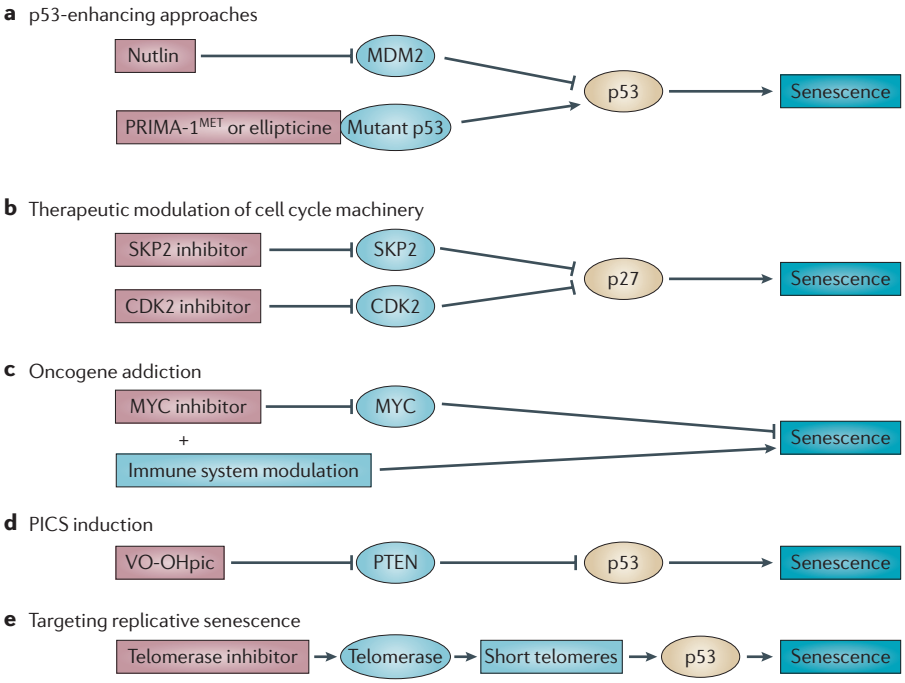


Figure 2 | **Strategies for the therapeutic activation and enhancement of senescence.** Schematic view of the approaches that are readily available for the implementation of pro-senescence therapy in cancer treatment. Inhibitors are shown in red boxes and target proteins in blue ovals. **a** | Enhancement of p53 activity through either inhibition of the interaction between MDM2 and wild-type p53 (for example, a nutlin) or restoration of mutant p53 activity (for example, PRIMA-1^{MET} or ellipticine). **b** | Modulation of cell cycle machinery, for example through either inhibition of S phase kinase-associated protein 2 (SKP2) or cyclin-dependent kinase 2 (CDK2). Both approaches result in increased p27 (also known as KIP1) activity and a consequent senescence response. **c** | Induction of senescence in tumours that are addicted to MYC through the inhibition of the MYC oncogene in combination with an immunomodulatory approach. **d** | Induction of PTEN loss-induced cellular senescence (PICS) through inhibition of PTEN and consequent mTOR-mediated activation of p53. **e** | Induction of replicative senescence through inhibition of telomerase and subsequent telomere shortening.

Therapeutic modulation of cell cycle machinery. The CDKIs INK4A and p27 have well-established and important roles in inducing senescence[88]. Although the induction of INK4A seems to be predominantly driven by transcription factors, the expression of p27 is strongly controlled through a balance of translation and proteasome-mediated degradation[89]. As detailed above, cells that lack *SKP2* are sensitized to senescence induction partly through the accumulation of p27, even in the absence of an intact p53 pathway[55]. A SKP1–CUL1–F-box protein (SCF)–SKP2 complex inhibitor (MLN4924) is currently in Phase I clinical trials, and it also now offers the potential to act as a pro-senescence therapy, through its ability to stabilize p27 (REF. 55) (FIG. 2b). In addition, it should be noted that although INK4A is frequently mutated in cancer, p27 is rarely mutated, but reduced levels and mislocalization of p27 correlate strongly with poor prognosis[90]. It has also recently been reported that in the context of MYC overexpression, the inhibition of CDK2 activity can promote senescence induction[69]. There are several pharmacological CDK inhibitors that can target CDK2 already in clinical trials as therapeutic agents[91], and their use as pro-senescence modalities may also prove to be beneficial as a cancer treatment (FIG. 2b).

Oncogene addiction. The role of MYC itself in promoting tumorigenesis has made a large contribution to the paradigm of oncogene addiction. Models of MYC overexpression demonstrate a poorer prognosis in response to chemotherapy when combined with the inactivation of key senescence pathways[92]. Importantly, senescence resulting from MYC inactivation requires an intact immune system, as previously outlined[82]. Therefore, the development and use of small molecules to inactivate MYC, to either target the protein for degradation or inhibit its activity (such as, 10058-F4 and its derivatives[93–96]) could also prove to be an efficient pro-senescence therapy. This strategy could be combined with an immuno-modulatory approach (FIG. 2c).

Table 1 | **Potential senescence-inducing small molecules in development**

Function	Compound	Stage of development	Refs
p53 stabilization	R7112 (nutlin-3 analogue)	Phase I	Recruiting: ClinicalTrials.gov identifiers NCT00559533 and NCT00623870
Mutant p53 reactivation	PRIMA-1^{MET} (APR-246)	Phase I	Completed: NCT00900614
	Ellipticine	Preclinical	87
SCF–SKP2 complex inhibitor	MLN4924	Phase I	Recruiting: NCT00722488, NCT01011530, NCT00677170 and NCT00911066
CDK inhibitors	Flavopiridol	Phase I/II	ClinicalTrials.gov*
	UCN-01	Phase I/II	ClinicalTrials.gov*
	CYC202 (seliciclib)	Phase I	Recruiting: NCT00999401
		Phase II	Terminated: NCT00372073
	SNS-032 (BMS-387032)	Phase I	Active (not recruiting): NCT00446342 and NCT00292864
MYC inhibitors	10058-F4 and its derivatives	Preclinical	93–96
PTEN inhibitor	VO-OHpic	Preclinical	39
Telomerase inhibitor	GRN163L (Imetelstat)	Phase I/II	ClinicalTrials.gov*

CDK, cyclin-dependent kinase; SCF, SKP1–CUL1–F-box protein; SKP2, S phase kinase-associated protein 2.*Several associated clinical trials can be found on the ClinicalTrials.gov website (see Further information).

PICS induction. Targeting of PTEN to elicit a senescence response is a provocative hypothesis that has been shown to be successful in *in vitro* and *in vivo* models[39]. At first glance, targeting such a potent tumour suppressor seems to be somewhat risky, particularly given the potential for the activation of PI3K–AKT signalling. However, there is clear evidence that many tumours present with mono-allelic loss of *PTEN*, especially at early onset[97], making the tumour particularly sensitive to PTEN inhibitors. As tumour cells expressing PTEN from only one intact allele have reduced levels of PTEN, the transient use of PTEN-targeting drugs, such as VO-OHpic, can completely, albeit transiently and reversibly, ablate its activity (FIG. 2d). This temporary and selective inactivation of PTEN activity in the tumour cells induces a senescence response through a signalling short circuit that is driven by hyperactivation of a PI3K–AKT–mTOR–p53 signalling pathway, as described above. By contrast, the effect of such inhibitors on wild-type cells that express PTEN at normal levels, results in only a transient decrease in PTEN activity leading to a marginal increase in the activation of the AKT–mTOR signalling pathway.

Interestingly, on the basis of our recent mechanistic findings for nuclear PTEN and INK4A upregulation in PICS[43], we would expect that VO-OHpic treatment would trigger p53 induction, but that upregulation of INK4A might not ensue owing to the remaining non-catalytic activity of heterozygous PTEN towards the APC/C–CDH1 complex. However, our data clearly indicate that the inhibition of the catalytic activity of PTEN through the use of VO-OHpic can still drive senescence[39], in spite of this potential drawback. This in turn suggests that PICS could be further enhanced through the potentiation of the INK4A arm of the response.

Targeting replicative senescence. Replicative senescence also offers the potential to be harnessed as a pro-senescence strategy. It has been reported that the reactivation of the telomerase complex, which is normally silenced in somatic cells, is required for the transformation process and progression of cancer[98,99]. There are several therapeutic approaches currently proposed that focus on targeting the telomerase complex[100]. Among the different approaches proposed, it is the specific inhibition of the enzymatic activity of telomerase that may represent a powerful pro-senescence approach. Indeed, the development of the inhibitor GRN163L, which is currently in Phase II clinical trials, holds promise as a strong anticancer agent, and its propensity to induce senescence should also be evaluated in this context (FIG. 2e).

Implementation in the clinic

Each of the strategies described above may be viewed as an independent approach to senescence induction and treatment of cancer. However, we should also consider the combination of pro-senescence strategies with already established treatment protocols and be mindful to incorporate pro-senescence approaches in the development of other novel therapies. For example, both neoadjuvant and adjuvant therapies have important roles in the treatment of cancers, including breast, prostate and colon cancer, where they significantly increase the disease-free survival and the overall survival of affected patients.

In a neoadjuvant setting, a pro-senescence approach could be combined with traditional treatments in order to reduce tumour mass before surgery. For example, VO-OHpic or nutlin-3 may be used in combination with radiotherapy in cancer patients who are not immediately suitable for surgery because of their age or advanced stage of disease at presentation. This may be particularly beneficial in patients with prostate cancer in which the vast majority of prostate cancers show decreased levels of PTEN at presentation but maintain an intact p53 response[101]. We believe that such a treatment may be expected to have two potentially positive outcomes. First, the induction of senescence itself may reduce tumour growth and trigger the immune system to clear senescent cells, contributing to the reduction of tumour burden. Second, as both senescence and apoptosis responses share key effector molecules (such as p53), the combination of pro-senescence approaches with traditional chemotherapeutic and radiotherapeutic protocols may have the added effect of tilting the balance of signalling towards apoptosis in those cells that are en route to becoming senescent.

However, it is also possible that cell cycle arrest that is triggered by a pro-senescent therapy could antagonize the apoptotic response to standard therapies.

The engagement of a pro-senescence response as part of an adjuvant treatment may also be beneficial. For example, it has been recently reported for a xenograft breast cancer model that the combination of ionizing radiation with a poly(ADP-ribose) polymerase (PARP) inhibitor results in a potent induction of senescence and consequent inhibition of tumour growth[102]. Furthermore, we propose that a PICS-type pro-senescence approach may be particularly advantageous in the adjuvant setting, as it may have the ability to target quiescent cancer cells more efficiently than either chemotherapy or radiotherapy. Thus, this treatment may reduce the statistical risk of relapse from occult disease (for example, residual disease in lymph nodes or systemic micrometastasis) that may arise from remaining quiescent CICs.

Conclusions and future directions

Accumulating *in vivo* evidence now demonstrates that senescence has an important part to play in the natural physiological response to tumour development[13]. However, we feel that the importance of senescence has been insufficiently recognized and that senescence induction for therapeutic benefit remains underexploited. Although we may be unknowingly promoting senescence with current therapeutic protocols, we think that the time has now come to take advantage of these intrinsic senescence pathways and to specifically enhance senescence for the potential eradication of disease through targeted approaches.

In developing novel approaches for pro-senescence therapy, such as the targeting of the SCF–SKP2 complex[55], we propose that the ability to promote senescence induction downstream of p53 and RB will have a substantial therapeutic effect, especially given the frequent mutations that inactivate these pathways. Furthermore, gene discovery should reveal novel biomarkers for senescence, and additional key players promoting the irreversible nature of this response. It is also now emerging that non-coding RNAs can also contribute to the senescence programme. For example, miR-20a has the ability to promote senescence induction through the downregulation of lymphoma-related factor (LRF; also known as ZBTB7A) and subsequent induction of ARF[103].

In addition to the identification of novel senescence pathways and players, it is also likely that novel types of senescence will be discovered. As is the case for PICS and OIS, new molecular types of senescence may demonstrate distinct mechanisms of activation that are amenable to therapeutic intervention.

Furthermore, the harnessing of senescence for therapy could make an important contribution to cancer prevention and could impede progression to advanced and metastatic disease. The ability to measure senescence *in vivo* and to identify pre-malignant lesions has the potential to allow the early detection and treatment of these lesions with pro-senescence modalities. As described above, we think that the use of a targeted pro-senescence therapy might minimize toxicity and enhance quality of life for cancer patients undergoing treatment. Although we can still learn much from *in vivo* modelling and *in vitro* studies, we believe that pro-senescence therapy has come of age. With the tools at hand, it is now the time to focus on the role of senescence in human cancer and to translate what has been uncovered to date to a clinically relevant context.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary
GRN163L | MLN4924 | PRIMA-1^{MET}

FURTHER INFORMATION

Pier Paolo Pandolfi's homepage: http://www.bidmc.org/research/departments/medicine/divisions/genetics/pandolfilab.aspx
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