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Review

National Institute on Aging Workshop: Repurposing Drugs or Dietary Supplements for Their Senolytic or Senomorphic Effects: Considerations for Clinical Trials

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Abstract

Background: Cell senescence is implicated in numerous age-related conditions. Drugs and nutritional supplements developed for a variety of purposes kill senescent cells (senolytics) or suppress their secretions (senomorphics). There is interest in repurposing such drugs to treat or prevent age-related diseases. To date, only small-scale preliminary trials have been conducted.

Method: At a workshop convened by the National Institute on Aging in August 2019, academic, industry, and government scientists reviewed issues for phase II trials of potentially repurposable drugs, or dietary supplements, to assess benefits and risks of their senolytic (killing senescent cells) or senomorphic (altering senescent cells' phenotypes) effects in treating or preventing age-related conditions.

Results: Participants reviewed mechanisms and effects of cellular senescence, senolytics, and senomorphics of several classes and their potential role in treating or preventing disease, modulators of the senescence-associated secretory phenotype, needs for senescence markers, data and specimen resources, infrastructure for planning trials, and potential effects on outcomes in older patients with multimorbidity and polypharmacy.

Conclusions: Participants noted the importance of considering potential effects of candidate drugs on multiple aging outcomes. It is important to assess drugs' specificity for killing senescent cells and the balance between senolytic and cytotoxic effects. Markers of specific senescent cell types are needed to assess intervention responses. There are potential interactions with coexisting diseases and their treatments in older persons. Standardized measures could enhance comparisons and pooling of data. Additional characterization of human cell senescent phenotypes is needed for developing better and more specific senolytics and senomorphics.

Keywords: Cell senescence, Clinical trials, Geroscience

This workshop was the first in a series planned by the Division of Geriatrics and Clinical Gerontology to review options for early-phase clinical trials of interventions modulating fundamental aging processes. Cellular senescence was chosen because of recent scientific advances, including identification of several classes of drugs that could be repurposed for their senolytic or senomorphic properties, development of new compounds by biotechnology companies, and recent initiation of exploratory clinical trials (1).

Considerations for Therapeutic Strategies

Senescent cells, characterized by cell cycle withdrawal, relative resistance to apoptosis, a senescence-associated secretory phenotype (SASP), and several types of macromolecular damage and metabolic dysregulation, increase in many aging tissues (2). The SASP is enormously complex and involves more than 400 proteins, as well as prostanoids, ceramides, leukotrienes, and nucleotides, and varies by cell type (3,4). In addition to direct effects of the SASP, senescent cells also induce senescence in nonsenescent cells (5,6).

Evidence for the role of senescent cells in age-related disorders is accumulating (5,7). However, cellular senescence also plays a positive role in embryonic development, tissue repair, and wound healing, and can both inhibit and stimulate tumorigenesis (8).

Senescence-inducing signals can be rapid, but senescent phenotypes appear to mature over days and weeks, an important feature for the timing of interventions and treatment regimens. In cell

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cultures, it takes 2–6 weeks for senescence to become established and a complete SASP to be acquired. Accordingly, senolytics might be effective if administered intermittently, for example, once daily, every other week or once a month, depending on the rate of generation of senescent cells. Such a "hit-and-run" approach could reduce side effects and, if effective, provide stronger evidence that the drug effects are mediated through killing senescent cells rather than effects that require continuous drug presence.

A principal strategy in senolytic drug development is based upon senescent cells' ability to resist apoptotic stimuli, implying increased prosurvival and/or antiapoptotic defenses. The objective is to develop drugs that kill senescent cells by transiently disabling prosurvival networks, which defend senescent cells against their own SASP, which can cause apoptosis in nearby nonsenescent cells. Development of senolytics is focusing on such prosurvival networks, including ephrins (EFNB1 or 3), PI3Kδ, p21, BCL-xL, plasminogen-activated inhibitor-2, BCL-2 family members, or heat shock protein-90 (HSP-90) (9,10) as well as metabolic targets in senescent cells that differ from those in nonsenescent cells.

Because different senescent cell types depend on different pathways for survival, an optimal approach would be to identify drugs or drug combinations engaging multiple targets within these networks, some components of which are redundant. Hence, it may be necessary to interrupt more than one pathway to kill particular types of senescent cells. Killing at least one reservoir of senescent cells is beneficial even if it is not the one primarily causing a disease because these cells can no longer spread senescence.

First-generation senolytics such as dasatinib, quercetin, and fisetin, and other drugs considered for repurposing as senolytics were selected from drugs known to affect targets related to cell senescence and resistance to apoptosis (9,11). Senolytic activity was also assessed for life-span-enhancing compounds tested in National Institute on Aging's (NIA) mouse Intervention Testing Program and in regulators of autophagy (10). Additional senolytics are now being identified using high-throughput approaches such as drug library screens.

Because senescence-associated pathways influence other cellular processes besides apoptosis resistance, an important consideration in screening candidate senolytics is their specificity for killing senescent versus nonsenescent cells. This has been assessed by comparing lethality in mouse and human nonsenescent cells with radiation-induced senescent cells (9) or senescence due to defective DNA repair (10). Several compounds had partial specificity for senescent cell lethality. Senolytics designed to target networks affecting predominantly senescent cells may cause fewer adverse effects in vivo than drugs less specific to senescent cells, which may trigger off-target apoptosis of nonsenescent cell types, with more side effects (9).

Many drugs with senolytic properties were originally developed as anticancer drugs, targeting mechanisms shared by cancer cells and senescent cells. Among these drugs, those with a favorable profile of more specific senolytic effects versus broader cytotoxic effects were considered as candidates for treating conditions related to cell senescence. Excessive toxicity and low efficacy that limit the use of these compounds in cancer treatment may be of lesser concern when these agents are used as senolytics because senescent cells do not proliferate and, unlike cancer cells, do not need to be fully eradicated. Therefore, lower doses and intermittent treatment regimens may address toxicity and efficacy issues (12). However, human experience with potential senolytics is limited and potential benefits should be carefully weighed against possible detrimental effects, such as reduced cancer immunosurveillance, cell cycle reentry of senescent cancer cells, and impaired tissue repair and regeneration.

Senomorphics that suppress the SASP or SASP components without killing senescent cells present an alternative approach. A cell culture screen identified senomorphic compounds based on reduced senescence-associated beta-galactosidase-positive cells without diminution of cell numbers (10).

Senomorphics need to be administered continuously, and hence require better safety profiles. Given the diversity of the SASP in differing senescent cell types, any one senomorphic may not inhibit all SASP components in all senescent cell types. SASP components vary among senescent cell types and over time. They include proteins and peptides, prostanoids, ceramides, leukotrienes, nucleotides, exosomes, and cell membrane changes affecting immune clearance of senescent cells. The target specificity of senomorphics may be enhanced by understanding the biological significance of different senescent cell populations and the effects of their SASP components (4). This information is needed to assess their relative importance in the pathophysiology of different conditions, which could allow development of more selective compounds to target specific SASP components or cells for specific conditions or combinations of conditions.

Experience With Specific Classes of Senolytics

Navitoclax and Other BCL-2/BCL-xL Family Inhibitors

Some, but not all, types of human senescent cells depend for survival on the BCL-2 family member, BCL-xL (9). BCL family inhibitors and compounds that target the H/Mdm2-p53 interaction are anticancer drugs that activate p53 and drive cells to apoptosis. In animal models, navitoclax, a Bcl-2 inhibitor, prevented neurodegeneration (13) and inhibited atherosclerotic plaque formation and growth by eliminating senescent cells (14). An H/Mdm2 inhibitor, UBX0101, prevented cartilage deterioration in knee joints of a mouse model of osteoarthritis. In a phase I placebo-controlled trial, a single injection of UBX0101 into knee joints of 48 participants with osteoarthritis was well tolerated and safe (15). Compared to placebo, high doses of UBX0101 (1–4 mg) significantly reduced pain and markers of cellular senescence, with a positive trend toward improved measures function, but the study was not powered to assess effects on this outcome.

Clinical development of BCL family inhibitors as anticancer drugs was affected by dose-limiting thrombocytopenia due to clearance of mature platelets (16). This side effect can be managed by platelet transfusions. For their senolytic properties, BCL family inhibitors could be administered intermittently, limiting exposure to a couple of days. Also, BCL family inhibitor dosing might be reduced by "apoptotic priming," that is, concomitant administration of drugs such as Bruton's tyrosine kinase inhibitors (eg, Ibrutinib), to increase BCL dependence of senescent cells (17) and thus improve BCL inhibitor safety profiles.

HSP-90 Inhibitors

Cell culture studies showed that treatment of aged mesenchymal stem cells with the HSP-90 inhibitor geldanamycin significantly increased apoptosis of senescent mesenchymal stem cells without affecting nonsenescent cells. In an animal model of cellular senescence, the HSP-90 inhibitor 17-DMAG (alvespimycin) removed senescent cells. Likewise, treatment of $Ercc1^{-/\Delta}$ mice with 17-DMAG reduced expression of SASP markers IL-1β, TNFα, and p16 and alleviated intervertebral disc pathology. Preclinical studies indicate that HSP-90 inhibitors' senolytic effects are mediated by targeting the

mitochondrial isoform of TRAP1 (tumor necrosis factor receptor associated protein 1) and the HSP-90-cdc37 complex. These drugs, alone or in combination with other senotherapeutics, could reduce the number of senescent cells in different tissues (10).

HSP90 inhibitors are used as anticancer drugs to target numerous proteins important for cancer cell survival and proliferation. Geldanamycin and radicicol are naturally occurring HSP90 inhibitors, but hepatotoxicity and structural instability limit their clinical utility (18). 17-DMAG development was discontinued due to neuro- and nephrotoxicity, hematologic malignancy and incomplete bone marrow recovery in the early-phase clinical trials (19). Development of other geldanamycin analogs was suspended or terminated because of low activity or unacceptable side effects such as night blindness and severe diarrhea (20–22). Rodent studies suggest that prolonged retinal inhibition by HSP-90 induces photoreceptor cell death, which is reversible upon discontinuation of the drugs (23).

There are some encouraging early testing results of several non-geldanamycin analogs. However, these agents also showed dose-limiting toxicity and low efficacy in subsequent oncology-based trials (24,25). PU-H71, a purine scaffold that binds the N-terminal ATP-binding domain of HSP90, showed significant activity and was well tolerated in a phase I clinical trial conducted by the National Cancer Institute (NCI). The trial was stopped due to lack of drug supply (26). Overall, ocular and liver toxicities, diarrhea, and fatigue limit clinical utility of HSP-90 inhibitors.

Dasatinib

Dasatinib, a pan-tyrosine kinase inhibitor, was approved by the U.S. Food and Drug Administration (FDA) in 2006 for treating imatinib-resistant chronic myelogenous leukemias and acute lymphoblastic leukemias that are positive for Philadelphia chromosome. It is well absorbed and rapidly metabolized by the P450 enzyme CYP3A4, and relatively well tolerated, with toxicities reversing after dose interruption.

Dasatinib alone or in combination with quercetin (see below) is senolytic in several cultured cell types, including myofibroblasts, pancreatic β cells, embryonic fibroblasts from Ercc1-deficient mice, and human primary lung fibroblasts (9,27). Administration of dasatinib to cultured mesenchymal stem cells from women with preeclampsia eliminated senescent cells (28).

When used continuously as an anticancer drug, dasatinib's common toxicities are myelosuppression, hemorrhage, fluid retention including pleural effusion, and adverse cardiac events. There were no differences in pharmacokinetics, efficacy, or safety between older or younger individuals. However, participants 65 years of age and older were more likely to experience fatigue, dose-dependent pleural effusion, dyspnea, congestive heart failure, and weight decrease. There were also post-marketing reports of atrial fibrillation and atrial flutter in patients treated with the drug (29).

In 130 patients from phase I–III clinical trials of dasatinib, 72% with chronic myelogenous leukemia had grade 2–4 neutropenia and/or thrombocytopenia. The median duration to onset of cytopenia was 27 days (30). In long-term dasatinib treatment, median time to onset of pleural effusion was 114 weeks, and older patients were at higher risk (31). Given these durations, intermittent administration and/or lower doses of dasatinib when used as a senolytic might reduce the incidence and severity of these adverse events. Studies in a rat model of dasatinib-induced pleural effusion suggest that co-treatment with antioxidants might reduce the risk of pleural effusion (32).

Flavonoids such as quercetin and fisetin act on numerous biological processes, so it is difficult to attribute their effects exclusively to senescent cell elimination. Fisetin had been showed to target senescent cells by inhibiting pro-senescence effectors such p16 and p21 (11) and quercetin - by affecting PI3K and other kinases (33). Despite short half-lives (3 hours for fisetin), they alleviate multiple senescence-associated conditions in animal models, even if administered every few weeks. This "hit-and-run" effectiveness is more compatible with senolytic activity than off-target effects that depend on continuously occupying receptors or modulating biochemical pathways or enzymes. Quercetin was the first flavonoid identified as senolytic. It suppresses senescent cell viability more than proliferating cell viability. Fisetin is more potent than quercetin and significantly reduced senescence-associated-β galactosidase and several SASP factors in animal and human tissues (34). Other effects included decreased oxidative stress and increased antioxidant defenses. In laboratory animals, fisetin suppressed levels of $p16^{Ink4a}$, a senescence marker. This effect continued after fisetin withdrawal, supporting the notion that clearing senescent cells provides prolonged benefit. Administration to very old mice increased median and maximum life span by about 15%, lowered serum levels of liver enzymes, reduced lipid peroxidation, and increased glutathione levels (11).

In cell culture and in vivo studies, quercetin and fisetin have targets consistent with their senotherapeutic properties. They can be both senolytic and senomorphic. Fisetin and other flavonoids are neuroprotective in multiple species and disease models, and fisetin is beneficial in animal models of diabetes, asthma, and cancer (35). In laboratory animal studies, no adverse effects were observed at fisetin doses as high as 3 g/kg per day for a month.

Fisetin and quercetin are rapidly conjugated, complicating analysis of bioavailability. Deconjugation can occur at sites of inflammation, resulting in localized release, which may contribute to therapeutic benefits. Both quercetin and fisetin reversibly inhibit cytochrome P450 2C8, which can affect the bioavailability of other drugs (36).

Both compounds are abundant in fruits and vegetables, and dietary consumption increases circulating levels. High ingestion correlates with lower serum lipids and decreased mortality from coronary heart disease (37). There is little evidence of adverse effects of fisetin and other flavonoids in humans, but controlled clinical trials are lacking. Safety and efficacy of flavonoids are being assessed in >60 clinical trials, mostly on neuroprotective effects of quercetin.

Dasatinib Plus Quercetin

The combination of these 2 senolytics affects different senescent cell antiapoptotic network nodes, and thus may kill a broader range of cell types than either compound individually. Thus, dasatinib kills senescent preadipocytes but not vascular endothelial cells, and quercetin vice versa. The combination of both drugs is more effective than either alone for killing certain senescent cell types (9).

In mouse models, dasatinib plus quercetin (D + Q) treatment cleared transplanted senescent preadipocytes, decreased frailty and age-related fat deposits, improved memory, and increased health and life span in old mice. (5). In mouse studies, D + Q also alleviated multiple senescence and age-related phenotypes, including bone loss (38), vasomotor dysfunction (39), and cognitive deficits (40).

There has been interest in potential benefits of D + Q for patients with idiopathic pulmonary fibrosis (IPF), a fatal disease that primarily affects people over age 60. Senescence markers accumulate in IPF patients' lungs, and higher expression of $p16^{INK4a}$, DNA damage

foci, telomere dysfunction, and SASP factors are associated with increased IPF severity (27). In a mouse model of bleomycin induced IPF, intermittent doses of D + Q alleviated senescent cell burden and improved physical function, body composition, pulmonary function, and exercise capacity (27).

In an open-label uncontrolled study in 14 IPF patients, D + Q was administered intermittently over 3 weeks to assess safety and feasibility of further studies. Participants reported one serious adverse event deemed unrelated to the intervention and several nonserious adverse events, including headaches, lung symptoms, and fatigue ranging in intensity from moderate to severe. Posttreatment gait speed, walking distance, and Short Physical Performance Battery score improved compared to pretreatment. However, since these measures are subject to learning effects, these results should be cautiously interpreted. SASP factor changes were moderately correlated with changes in physical function, pulmonary function, and an index of frailty (41).

A 2-week open-label uncontrolled study in 9 diabetic kidney disease patients (mean age 69) assessed a 3-day course of oral D + Q. Gastrointestinal and other symptoms, but no serious adverse events, were noted. The proportion of adipose tissue cells with senescence markers including p16, p21, and beta-galactosidase-expressing cells was reduced, as were several circulating SASP factors (42).

Selected Modulators of the SASP (Senomorphics)

Rapamycin

Rapamycin extends life span and health span in several laboratory animal models and has multiple cellular effects. It inhibits cellular senescence in a broad range of cells, but its mechanism is not fully established. Hepatocytes from mice treated with rapamycin for 6 months had increased levels of Nrf2, a protein regulating protection against oxidative damage triggered by injury and inflammation (43). Nrf2's role in mediating rapamycin's effects on cell senescence was clarified in rodent Nrf2 knockout studies: rapamycin-induced decreases in senescence markers p16 and p21 required Nrf2, but that rapamycin's diminution of beta-galactosidase and SASP did not (44).

Rapamycin was approved by FDA in 1999 to prevent transplant rejections and (in drug-eluting stents) to limit coronary artery restenosis. Three rapalogs are approved by the FDA for several indications. In human cell cultures, rapamycin restrained an inflammatory SASP arm by suppressing IL-1-α translation and dampened senescent cells' protumorigenic effects in mice (45).

Most human studies of systemically administered rapamycin and rapalogs involved transplant patients taking combinations of immunosuppressant drugs. Thus, interpretation of their safety is confounded. The most common side effects, affecting >20% of transplant patients, are stomatitis/mouth sores, rash, and hyperlipidemia. Rare (<5%) and very serious effects include pneumonitis and bone marrow suppression. There are also beneficial side effects in transplant patients, including fewer skin cancers, non-Hodgkin's lymphomas, viral infections, and reduced cardiac allograft vasculopathy.

Clinical experience with rapamycin and rapalogs in older adults without conditions for which the drugs are FDA-approved is limited. An uncontrolled pilot trial in 13 cardiac rehabilitation patients (mean age 74 ± 8 years) tested safety and tolerability of 0.5–2.0 mg rapamycin daily (46). Rapamycin was relatively well tolerated, but 62% of participants developed diarrhea. A placebo-controlled trial tested effects of 1 mg rapamycin administered for 8–16 weeks in 28 older adults 70–95 years of age (47). Healthy persons and individuals with stable chronic diseases, such as hypertension, asthma, and coronary artery disease, were eligible for the study. Small decreases in hemoglobin, hematocrit levels, red cell count, and body weight in the rapamycin group differed significantly from changes in the controls, but the clinical importance of these changes is uncertain. Circulating levels of numerous cytokines including SASP components were not significantly affected by rapamycin, except for an increase in TNF-alpha. Two participants taking rapamycin and one taking placebo developed stomatitis. Another 2 taking rapamycin discontinued the study, one due to diarrhea and one due to facial rash. Larger controlled trials of the rapalog everolimus (RAD001) and the mTOR inhibitor dactolisib (RTB101) found improved responses to influenza vaccination and prevention of infections in older persons (48,49). In these trials, study drugs were relatively well tolerated, but with higher incidence of adverse effects including mouth ulceration and increase in serum cholesterol. There are no data from any of the above human studies on in vivo effects on cellular senescence phenotypes, for example, p16 or p21 activity.

Janus Kinase Inhibitors

The Janus kinase-signal transducer and activator of transcription (JAK/STAT) pathway regulates cell proliferation, differentiation, migration, apoptosis, and production of pro- and anti-inflammatory cytokines, growth factors, and many metabolic cytokines. The 4 species of Janus kinases are differentially affected by differing JAK inhibitors.

In vitro, JAK inhibitors significantly reduced several SASP cytokines, including IL-6, MCP-1, MCP-3, and GM-GSF, in human preadipocytes and vascular endothelial cells made senescent by irradiation or serial passage, but not in nonsenescent cells. In vivo, markers of JAK/STAT pathway activity, cell senescence, and inflammatory SASP components are higher in adipose tissue of old mice compared to young mice (50). Administration of 60 mg/kg ruxolitinib for 2 months to 24-month-old mice significantly decreased levels of circulating inflammatory markers, reduced inflammation in adipose tissue, and age-related fat loss. The JAK inhibitor improved bone phenotypes (38) and metabolic function (51), including blood and hepatic triglyceride levels, circulating free fatty acids, and insulin sensitivity. Mice receiving ruxolitinib had higher activity levels and greater endurance, grip strength, and walking speed (50). In young mice, ruxolitinib had no effect on bone phenotypes, and physical or metabolic function (38,50,51).

JAK inhibitors are FDA-approved for several conditions: ruxolitinib for myelofibrosis and polycythemia vera, tofacitinib for rheumatoid and psoriatic arthritis, and baricitinib for rheumatoid arthritis. In a clinical trial of ruxolitinib for myelofibrosis, the most common adverse events were anemia, neutropenia, and thrombocytopenia (52). These effects could be either species- or disease-specific, because in mice they have not been observed (50).

Data regarding the potential therapeutic role of JAK inhibitors as senomorphics are limited. The extent to which their therapeutic benefits are mediated by effects on senescent cells versus nonsenescent cells is unclear. In addition, benefits and risks of long-term treatment, which might be required for delay or arrest of aging-related conditions, have not been studied. It is also unclear which SASP components contribute to specific conditions and might be individually targeted, which could provide more favorable benefit-risk profiles. The potential effects of combining JAK inhibitors with other drugs

that inhibit the SASP, such as metformin and rapamycin, have also not yet been tested.

Biomarkers of the Presence and/or Phenotype of Senescent Cells in Tissues

In vivo human biomarkers of cell senescence will be crucial in phase II trials of senolytics and senomorphics for identifying intervention target populations with high senescent cell burdens, assessing interventions' degree and specificity of target engagement, and determining their ability to eliminate senescent cells and/or modulate their phenotypes. In drug repurposing studies, such markers can also guide development of new senolytic or senomorphic drugs with improved efficacy and safety.

Cellular senescence markers are associated with aging-related human diseases, including Alzheimer's disease, Parkinson's disease, atherosclerosis, and premature coronary artery disease (2). They have been assessed in several issues and cell types that could be measured in human intervention studies. Reduced gene expression for SASP components in skin cells correlated with clinical improvement in a small single-arm trial of dasatinib in systemic sclerosis patients (53). In vitro, abdominal fat mesenchymal stem cells from women with preeclampsia have increased senescence-associated beta-galactosidase expression and upregulation of SASP components, compared to cells from controls (28). Senescent cell burden and phenotype, manifested as p16, p53, IL-6, and MCP-1 gene expression, were upregulated in cells from obese subjects compared to controls (54).

Common cellular senescence measures, such as senescence-associated beta-galactosidase and $p16^{INK4a}$, are not specific to senescent cells. Since no individual marker is completely senescence-specific, and senescent phenotypes vary among tissues, a combination of cellular markers has been recommended for quantifying and characterizing senescent cells in individual human tissues (2).

Studies of 3 senescence inducers: X-ray irradiation, oncogenic RAS overexpression, and atazanavir (a protease inhibitor used for HIV-AIDS), identified a core of SASP proteins shared among all 3 inducers and among different cell types. Some of these are deleterious and some beneficial (4). These findings are pertinent to studies of circulating factors that could be systemic indicators of SASP activity in humans. In human plasma, circulating levels of several SASP proteins, including GDF15, STC1, SERPINs, and MMP1, varied significantly with donor age (4,55).

Animal model studies are profiling different cell populations using RNA-seq to generate senescent cell associated signatures for subpopulations of senescent cells and core signatures across different senescent cell types. These senescence-associated signatures could identify senescent cell subpopulations and aid in discovery and development of senolytics to target them.

Senescence signatures depend upon the inducers of senescence, cell type, and the duration after induction of senescence when phenotypes are assessed. Replicative senescence, oncogene-induced senescence, and ionizing radiation-induced senescence up- or downregulate create differing stress-dependent signatures. A set of genes at the core of the senescent phenotype is independent from the senescence-inducing stressor, thus creating stressor-independent signatures. Four senescent cell types: fibroblasts, melanocytes, keratinocytes, and astrocytes, vary significantly in gene expression, allowing identification of cell type specific senescence-associated signatures. However, a core of 55 genes were dysregulated regardless of cell type (56). Notably, expression of many senescence-related genes is independent of the time since senescence induction, while expression of others is time-dependent, indicating that the senescence phenotype develops dynamically.

Resources and Research Issues for Future Trials

While interventions targeting cell senescence and other aging mechanisms have been tested in animal models, human studies have not yet assessed the extent to which targeting such mechanisms could delay, prevent, or alleviate multiple age-related disorders. Trials to assess such interventions face different challenges than the traditional paradigm of one drug-one target-one disease.

Translational Geroscience Network

To address such challenges, the Translational Geroscience Network (TGN), supported by NIA, will provide infrastructure and analyses for phase I or II trials on conditions related to one or more aging mechanisms. For ethical and practical reasons, its initial focus is symptomatic conditions whose patients could benefit directly from treatment, particularly those with poor current treatment options. Target populations could include persons with multiple coexisting morbidities (with treatment effects assessed for each), or conditions with features of accelerated aging (eg, cancer in posttreatment survivors, HIV, obesity-related diabetes, and progeroid syndromes). Trials targeting cell senescence could also address tissue-specific conditions that could be treated by local drug administration, such as osteoarthritis or skin conditions. Trials could also address age-related impairments in responses to stressors such as infections or surgery. After efficacy and safety are assessed in such studies, clinical trials could expand to prevention studies in individuals at risk for such conditions.

The TGN will develop standard operating procedures, clinical and assay protocols, and a suite of biomarkers that can be used across trials to assess drug effects on multiple aging mechanisms and clinical outcomes and allow data compilation from multiple studies. A suite of biomarkers across trials is also valuable for assessing the effect of targeting one aging process on other aging processes. The TGN will provide biostatistical consultation, biobanking, and assistance with meeting FDA regulatory requirements. It will also coordinate efforts across multiple institutions and serve as a training and information exchange resource.

Analyses of Data and Specimens From Previous or Ongoing Studies

Analyses of data or specimens from previous trials of compounds being considered for repurposing as senolytics or senomorphics could inform the designs of trials to test their potential for this use, for example, by identifying additional affected pathways or phenotypes. Such analyses have not yet been done for data or specimens from cancer treatment trials of senolytics. Cancer databases and repositories could be valuable in assessing potential effects of senolytic or senomorphic drugs. The Cancer Genome Atlas (TCGA) project, supported by the NCI, collected and analyzed over 20 000 biospecimens from 11 000 cancer cases from multiple cancer types. It can provide clinical, pharmacologic, pathology, genetic, and molecular data from individuals from these studies (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga).

Such data can be analyzed to assess the relationships of various SASP elements to differing phenotypes, drug effects, and clinical outcomes. Other cancer-related sources of data and specimens include the NCI's National Clinical Trials Network and Cancer Moonshot Biobank.

Data and specimens from clinical trials of interventions that may affect aging mechanisms, for example, the Diabetes Prevention Program trial of metformin and lifestyle interventions, could be analyzed to assess treatment effects on cell senescence or SASP components, and relationships of these effects to clinical outcomes and other aging mechanisms. Analogous analyses could be conducted on specimens and data from observational cohort studies. For studies with genetic data but lacking biospecimen molecular data (eg, the UK Biobank), Mendelian randomization methods could be used to infer SASP phenotypes or aging-related molecular changes for which genetic relationships have been established.

Drug Interactions

Older potential persons who may be candidates for senolytic or senomorphic treatment often have multiple coexisting conditions and take multiple medications. Thus, it is important to consider senolytic and senomorphic drug–drug interactions (DDIs) as well as drug–disease interactions.

A key DDI type consists of effects of one drug on clearance or transport of others. FDA requires information on substantial pharmacokinetic DDIs in its drug approval process. Hence, there is considerable DDI information on FDA-approved drugs considered for repurposing as senolytics. Ruxolitinib, dasatinib, navitoclax, and rapamycin are metabolized by the cytochrome P450 CYP3A pathway and interact with numerous other drugs that inhibit or induce this pathway. Dasatinib's gastric absorption is pH-dependent and can thus be affected by use of antacids or proton pump inhibitors. Several JAK inhibitors decrease creatinine clearance (57), suggesting possible pharmacokinetic effects on other drugs dependent on renal excretion.

Less pharmacokinetic DDI information is available on flavonoids such as quercetin or fisetin (which also is metabolized by CYP3A). For these and other potential senolytic/senomorphic compounds not approved by FDA, in vitro and in vivo testing of interactions using approved enzyme and transporter index substrates and inhibitors will be important (58).

Pharmacodynamic interactions of senolytics or senomorphics may be at least as important as pharmacokinetic interactions. Clinical pharmacology studies on possible adverse effects of interactions with drugs used widely by older persons will be important for deciding whether concurrent use is safe, warrants caution, or should be contraindicated in differing patient groups. Rapamycin-related decreases in hemoglobin and hematocrit (47) indicate a need to assess safety in patients using anticoagulant and antiplatelet drugs. Dasatinib-induced reduction in platelets may increase bleeding risks in the many patients with cardiovascular disease and other conditions who use these drugs. Its effects on fluid retention can be aggravated by nonsteroidal anti-inflammatory drug (NSAID) use and could raise risk in the many older heart failure patients who use NSAIDs for osteoarthritis. Dasatinib's prolongation of cardiac repolarization could also be especially risky in patients taking drugs with action potential repolarization effects such as ranolazine.

The extremely high prevalence of polypharmacy (eg, ≥5 drugs) among older persons poses a high likelihood of drug interactions with senolytic or senomorphic drugs. The diverse drug combinations found in older patients may contribute to unexpected interactions that would not be found in studies on interactions with a single drug or drug-metabolizing enzyme. A trial of rapamycin in relatively healthy older adults found substantial polypharmacy, even though persons taking drugs affecting CYP3A activity were excluded (47). The high degree of polypharmacy reflects the high prevalence of multiple coexisting morbidities and risk factors in older persons. Multimorbidity, independent of DDIs, may pose challenges for senolytic or senomorphic drug use. Thus, for example, information is needed on the extent to which lowering hematocrit and hemoglobin levels by rapamycin could worsen subclinical anemia, which is common in older adults. The increased risk that JAK inhibitors pose for herpes zoster and other infections (57) should be considered regarding their repurposing as senomorphics, particularly in older persons with age-related impairments in immune function.

A key issue for senolytic therapy is the extent to which intermittent versus chronic administration could lessen adverse drug interactions. Data are also needed on risks and benefits of temporary suspension of drugs that interact adversely with senolytics during intermittent senolytic administration. Pharmacokinetic data on elimination of drugs will likely be insufficient to address this question completely, as many drugs' clinical effects persist well after they are fully cleared.

Additional Biological and Clinicopathological Information Needed for Clinical Trial Design and Analysis

Additional information on human cell senescence and its relationship to clinical outcomes will be crucial for optimizing design of future phase I and II trials of senolytics and senomorphics and interpreting their results. Human studies including (but not limited to) early-phase intervention studies could contribute such data, which could address the following trial design issues.

Senescent Cell Types and/or SASP Components to Target

Diverse senescent cell types can be characterized by differing cellular markers and SASP components. Establishing and validating a marker panel to distinguish senescent cell types in different tissues could clarify their relationships to existing clinical conditions and risk of future events. These markers could also be used to assess the degree and specificity of killing or phenotype modification by senolytic and senomorphic drugs.

Selecting Clinical Trial Target Populations and Methods to Screen Potential Participants

The above information could be used to identify relationships of senescent cell burden to disease severity and risk, needed to identify individuals with high burden who might present the most favorable ratio of potential benefits to risks from senolytic or senomorphic treatment.

Determining Schedule of Senolytic Drug Treatment

Potential intermittent "hit-and-run" senolytic drug therapy poses the challenge of assessing the rate of re-accumulation of senescent cells after a treatment episode and the relationship of re-accumulation to changes in clinical status. This will likely require development of specific senescence markers that can be assessed repeatedly in an individual.

Feasible Measures of Senescent Cell Type, Burden, and Target Engagement in Clinical Studies

The development of indicators of senescent cell type and burden for tissues that cannot be feasibly sampled (or sampled repeatedly) in trial screenees or participants poses a challenge. This problem could be addressed if correlations could be found between senescent cell burden (or changes in burden) in such tissues and levels (or changes in levels) of SASP markers in blood (eg, IL-1α, IL-2, MMP-2, and MMP-9) or with senescence markers in feasibly sampled tissues such as skin or subcutaneous fat (eg, p16, p21, or Sa-βgal).

Measures of Treatment Responses

Because senolytics and senomorphics affect mechanisms besides cell senescence, assessing favorable and unfavorable off-target effects will be important. Measures of these effects could also facilitate analyses of the extent to which treatment effects on the primary outcome are mediated by effects on cellular senescence or by other mechanisms. In addition, given senescent cells' presence in multiple tissues, trials of systemically administered senolytics or senomorphics should assess effects on multiple aging-related changes and risk factors, and indicators of other aging mechanisms influenced by cell senescence.

Potential Benefits of Standardization of Measures

Comparing results of senolytic and senomorphic interventions across trials and pooled data analyses where appropriate would advance understanding of senolytic and senomorphic drug effects. This will require a core set of protocols for specimen storage, assays, and quality control procedures that could be shared among studies or conducted by a central laboratory for multiple studies. Such shared outcome measures for multiple aging-related phenotypes and risk factors, and indicators of the status of putative aging mechanisms, would increase understanding of the breadth of impact of senolytic and senomorphic interventions.

Conclusion

Key considerations from the workshop, discussed in the preceding sections, are presented briefly in Table 1.

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Conflict of Interest

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Table 1. Workshop Conclusions

- Several drugs and other compounds kill senescent cells (senolytics) or suppress their senescence-associated secretory phenotypes (senomorphics).
- Cellular senescence is implicated in numerous age-related conditions. Planning for repurposing a drug with senolytic or senomorphic effects should consider potential impacts on more than one condition.
- Because components of senescence-associated pathways affect other processes, candidate drugs' specificity for senescent vs nonsenescent cells should be assessed.
- Anticancer drugs with favorable profiles of senolytic effects vs broader cytotoxic effects may be promising candidate senolytic treatments.
- Because senescent cells do not proliferate and do not need to be fully eradicated, lower doses and intermittent treatment in repurposing cancer drugs as senolytics may reduce toxicity, but benefits need to be weighed against potential risks.
- Senomorphic drugs that do not kill senescent cells would need to be administered continuously, and hence require better safety profiles.
- Analyses of data or specimens from previous trials of compounds considered for repurposing as senolytics or senomorphics could inform trial designs by identifying affected pathways or phenotypes.
- Markers to distinguish senescent cell types in different human tissues are needed to clarify their relationships to differing conditions that could be targeted in clinical trials.
- Markers of specific senescent cell types that can be assessed repeatedly are needed in clinical trials to screen for individuals with high senescent cell burden, assess target engagement, measure elimination of senescent cells or modulation of their phenotypes, and determine schedules of intermittent treatment by measuring rate of disappearance and re-accumulation of senescent cells.
- Validated circulating indicators of senescent cell type and burden are needed for tissues that cannot be feasibly sampled in trial screenees or participants.
- Given the prevalence of multimorbidity and polypharmacy among older persons, consideration is needed regarding senolytic and senomorphic drug–drug and drug–disease interactions, potential contraindications, and implications for drug scheduling.
- It is important to assess off-target effects, the extent to which treatment effects are mediated by effects on cell senescence, and effects on a variety of aging-related changes, risk factors, and other aging mechanisms influenced by cell senescence.
- Standardized measures across studies would enhance comparability of results of interventions and pooled data analyses to provide greater power for assessing intervention effects.
- Additional characterization of human senescent cell types and senescence-associated secretory phenotype components, and their physiologic and clinical effects, is needed for developing senolytic or senomorphic drugs with improved efficacy and safety and testing them in clinical trials.

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Author Contribution

All the authors equally contributed to writing the paper.

Disclaimer

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