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Perspective

Biomarker-Guided, Stage-Specific Senotherapy for Metabolic Dysfunction-Associated Steatotic Liver Disease

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ABSTRACT: Cellular senescence drives inflammation and fibrosis progression in metabolic dysfunction-associated steatotic liver disease (MASLD) through the senescence-associated secretory phenotype (SASP). Existing research covers hepatocyte types, SASP signaling pathways, and senescence-targeting drugs. However, basic mechanistic understanding and precise clinical translation remain disconnected. An integrative therapeutic framework that couples senescence heterogeneity with disease stage-specific microenvironmental contexts is still lacking. This Perspective presents a hypothesis-generating translational framework and a candidate sequential trial design. The proposed sequence would combine short-term senescent cell clearance with long-term senescence modulation and metabolic maintenance. By stratifying senescence biomarkers according to clinical decision functions into patient selection markers, treatment timing markers, and response efficacy markers, and by defining candidate therapeutic windows based on disease stages, this study proposes lifestyle intervention and senescence modulation as proposed preventive strategies during simple steatosis, the testing of short-term senescent cell clearance during metabolic dysfunction-associated steatohepatitis (MASH) and fibrosis within prospective trial settings, and careful evaluation of intervention feasibility and anticipated benefits under strict risk stratification during cirrhosis. Patients with active hepatocellular carcinoma or high-risk premalignant lesions should currently be excluded from early senolytic trials pending dedicated safety data, rather than being described as an absolute clinical contraindication. This work aims to provide an actionable and verifiable reference framework for precise translation of senescence-targeted therapy in MASLD, moving the field from whether it works toward for whom it works, when it works, and how to test it safely and effectively. All proposed thresholds, strategies, and sequences are candidate endpoints for prospective validation.

Keywords: cellular senescence, senescence-associated secretory phenotype, senotherapy, sequential therapy, therapeutic window

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide. It includes simple steatosis, metabolic dysfunction-associated steatohepatitis (MASH), progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. Preclinical and preliminary clinical evidence indicate that cellular senescence drives MASLD-related inflammation and fibrosis through the senescence-associated secretory phenotype (SASP) [2]. Most current senotherapeutic strategies, however, do not adequately account for differences in senescent cell types,

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spatial distribution, and microenvironmental contexts across MASLD stages [3]. Simple steatosis, nonfibrotic MASH, fibrotic MASH, and cirrhosis each show distinct senescent cell ecological features. The predominant sources and phenotypes of senescent cells vary, and the composition, intensity, and downstream effects of the SASP change qualitatively as disease advances [4]. A uniform intervention is unlikely to achieve equivalent efficacy across all stages. This homogenized approach may partly explain why several senotherapeutic strategies have fallen short in clinical translation.

The central argument is that effective senotherapy may require differentiated interventions based on disease stage and senescent cell characteristics. The proposed framework is that early disease warrants preventive senomorphic modulation, active inflammatory disease could be tested with short-course intensive clearance, fibrotic disease could be tested with clearance plus anti-fibrotic strategies, and cirrhosis requires individualized assessment grounded in immune microenvironment status, primarily within research settings. This work constructs a proposed translational research pathway from three dimensions: biomarker-guided patient stratification, treatment timing selection, and efficacy monitoring. On this basis, the sequential model combining short-term senolytic clearance with long-term senomorphic modulation and metabolic maintenance constitutes a testable scientific hypothesis and a candidate trial-design framework, providing a theoretical rationale and experimental design reference for subsequent validation.

2. Stage-Specific Therapeutic Windows

2.1 Senescent Cell Ecosystems Across Stages

The natural course of MASLD can be divided into four stages. Each stage shows marked differences in the senescent cell ecosystem and candidate therapeutic window, and these differences form the biological basis for stage-specific intervention [5]. Senescence is not a homogeneous cellular state. It is a spectrum of phenotypes that evolve with the tissue microenvironment. In actual MASLD tissue, senescent or stress-associated hepatocytes, hepatic stellate cells (HSCs), Kupffer cells, endothelial cells, and immune populations may coexist across stages. It is worth noting that this four-stage model is a conceptual simplification rather than an established biological classification. The proposed stage-specific dominance of particular cell populations requires validation by single-cell and spatial omics.

Simple steatosis is proposed to be characterized mainly by stress-associated or pre-senescent hepatocyte-like states with minimal SASP expression. The main sources of these stress responses are oxidative stress and lipotoxic stress from metabolic overload, and cell cycle regulatory pathways have not yet undergone irreversible changes [6]. Lipid droplet accumulation in hepatocytes leads to endoplasmic reticulum stress and mitochondrial dysfunction, which generate reactive oxygen species and trigger DNA damage responses [7]. Hepatocytes at this stage tend to show elevated p21 expression but relatively limited p16 expression. However, p21-high/p16-low status alone should not be automatically classified as established cellular senescence. These cells should be described as stressed, pre-senescent, or transiently arrested cells. Their partial reversibility remains a hypothesis requiring rigorous marker-based validation [8]. The candidate therapeutic window at this stage offers the highest potential reversibility. The proposed intervention is lifestyle modification, including caloric restriction, dietary optimization, and regular physical activity [9]. Senomorphic agents may be added for preventive purposes. The goal is to prevent irreversible conversion of stress-associated cells to pathogenic senescence phenotypes. Senolytics are not proposed for routine testing at this stage because senescent cell burden remains low and elimination may interfere with adaptive metabolic responses [10].

In nonfibrotic MASH (F0 to F1), a synergistic amplification loop emerges between senescent or stress-associated hepatocytes and activated Kupffer cells. Kupffer cells undergo proinflammatory polarization after uptake of free fatty acids and oxidized lipids. They release tumor necrosis factor-alpha and interleukin-1β, which exacerbate oxidative damage and senescence induction in hepatocytes [11]. A positive feedback loop forms. Hepatocyte senescence triggers SASP release, SASP activates Kupffer cells, and increased proinflammatory cytokines induce senescence in additional hepatocytes [12]. SASP drives substantial inflammatory responses without significant extracellular matrix deposition [13]. The candidate therapeutic window at this stage corresponds to the SASP-driven pro-inflammatory phase. Short-course senolytic clearance plus SASP modulation could be tested prospectively, with the goal of eliminating established pathogenic senescent cells and interrupting the inflammatory amplification loop [14]. Theoretically, the benefit-risk ratio at this stage is potentially optimal because fibrosis has not been established and hepatic tissue retains regenerative and remodeling capacity after senescent cell clearance.

Fibrotic MASH (F2 to F3) is dominated by pro-fibrotic persistent senescent HSCs and a multi-cellular senescence network. Activated HSCs enter senescence under sustained oxidative stress and inflammatory cytokine stimulation. Senescent HSCs retain the capacity to secrete extracellular matrix components. Tissue inhibitors of metalloproteinases and pro-inflammatory

factors in their SASP further maintain a fibrotic microenvironment [15]. HSC senescence has dual effects. Transient senescence can limit HSC proliferation through cell cycle arrest and suppress fibrosis progression. Persistent senescence drives disease exacerbation through the SASP under chronic metabolic and inflammatory stress. This duality imposes stringent requirements for therapeutic precision. The proposed, but unvalidated, intervention concept requires selective elimination of pro-fibrotic persistent senescent HSCs while preserving potentially protective transient senescent cell populations [16]. Extracellular matrix continues to accumulate, hepatic lobular architecture begins to remodel, and vascular resistance gradually increases [17]. The candidate therapeutic window at this stage is the anti-fibrotic combination therapy period. Senolytic agents and anti-fibrotic drugs could be tested together in future trials, with the goal of eliminating pro-fibrotic senescent HSCs and suppressing de novo collagen deposition.

Cirrhosis (F4) and HCC high-risk state manifest extensive senescence and immune exhaustion. Multiple liver cell types enter senescence. Senescent cell density reaches its peak, and immune surveillance function declines markedly [6]. The microenvironment undergoes profound reprogramming, and some senescent cells may evade immune clearance and persist within a pro-tumorigenic niche [18]. The relationship between senescence and immunity is complex at this stage. Persistent senescent cells consume immune resources and promote immune tolerance. Large-scale elimination of senescent cells may release substantial cellular contents and trigger excessive inflammatory responses [19]. The paradoxical role of senescence in hepatocarcinogenesis also matters. Senescence suppresses tumor initiation through cell cycle arrest, but SASP promotes established tumor progression. This mandates particular caution in any senescence intervention at this stage [14]. The therapeutic window narrows considerably. The feasibility of senolytic intervention requires careful evaluation under stringent patient screening, and anticipated benefits are more limited than in earlier stages. In the presence of high-grade dysplastic nodules or early HCC, senolytic clearance may paradoxically promote tumor progression. Therefore, patients with active HCC or high-risk premalignant lesions should currently be excluded from early senolytic trials pending dedicated safety data. This should not be framed as an absolute clinical contraindication for all future contexts, because the evidence base is insufficient for such a definitive statement (Fig. 1).

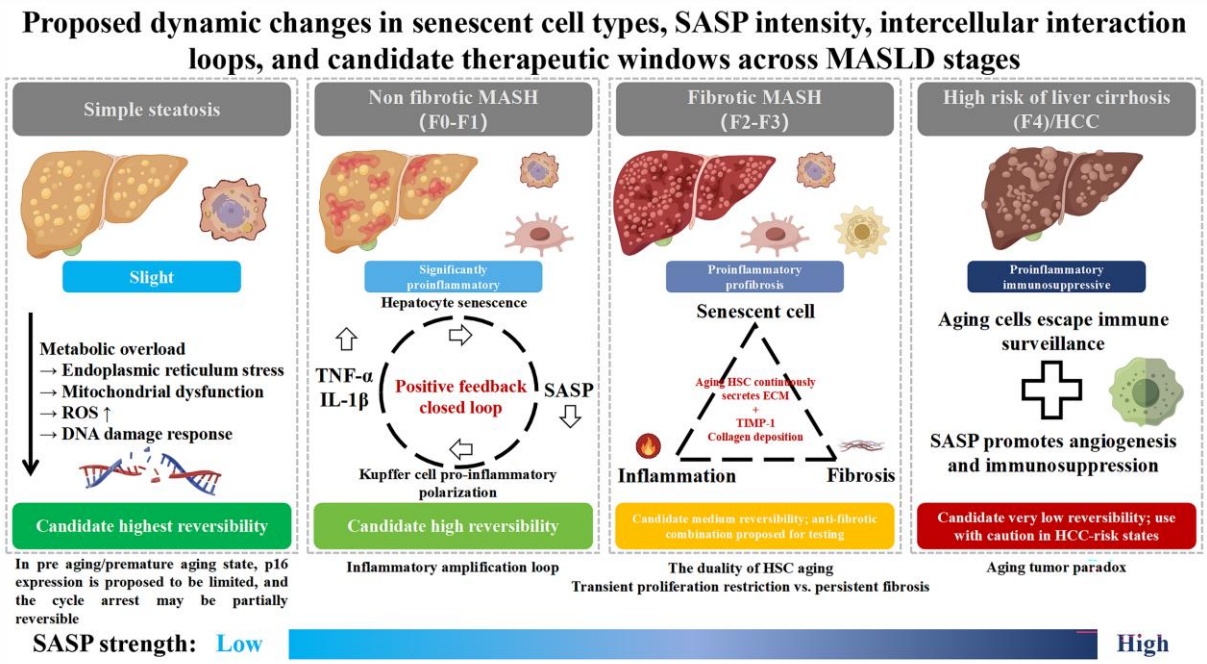


Figure 1. Proposed dynamic changes in senescent cell types, SASP intensity, intercellular interaction loops, and candidate therapeutic windows across MASLD stages. This figure is a conceptual model based on current literature and is intended to illustrate proposed stage-specific differences, not to present validated biological classifications. The four-stage model is a simplification; the proposed dominance of particular senescent cell populations, the depicted SASP intensities, and the reversibility estimates all require validation by single-cell and spatial omics. All therapeutic window labels (including "candidate highest reversibility," "candidate high reversibility," "candidate medium reversibility," and "candidate very low reversibility") are proposed for future testing and should not be interpreted as established clinical recommendations. HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; SASP, senescence-associated secretory phenotype.

2.2 Operational Definition of Therapeutic Windows

Translating therapeutic windows from conceptual descriptions to operational definitions requires three core parameters. These parameters are proposed for future trial design.

The temporal window refers to the candidate optimal time interval for initiating senescence intervention in each stage. Beyond this interval, the benefit-risk ratio of intervention may decline substantially. For patients with F2 to F3 fibrosis, the temporal window needs to consider both fibrosis progression rate and hepatic functional reserve. Patients with slow progression and well-preserved liver function may have a wider temporal window. Rapid progressors may require earlier intervention [20].

The cellular window denotes the senescent cell types that are proposed as primary intervention targets in each stage. The operational challenge is the current lack of clinically available biomarkers that distinguish senescent cells of different cellular origins. Single-cell transcriptomics may provide a technical basis for this purpose [10].

The molecular window refers to available biomarkers and their corresponding candidate decision thresholds in each stage. Importantly, FIB-4 and MRE are disease severity and fibrosis markers. They do not directly measure cellular senescence and should be separated from senescence-specific biomarkers. They may be used for disease staging and risk enrichment, but not as direct molecular measures of senescent cell burden.

These three parameters form the core content of biomarker-guided stage-specific therapy and represent an essential step in moving this framework from theory toward clinical implementation (Table 1).

Table 1. Therapeutic window parameters and proposed intervention strategies for future testing across MASLD stages.

Stage	Temporal window features	Cellular targets	Disease or fibrosis staging and candidate senescence biomarkers	Proposed strategy for future testing, not clinical recommendation	Benefit-risk assessment	Evidence level or status
Simple steatosis	Candidate highest potential reversibility; widest candidate window	Stress-associated or pre-senescent hepatocytes; senescent cell clearance not proposed	Low SEPP1 levels; MRI-PDFF; p21 and p16 IHC require cautious interpretation and are not established senescence-specific markers	Lifestyle intervention; optional senomorphic prophylaxis with metformin within research settings only	Preventive benefit hypothetically outweighs risk	Preclinical, observational, or hypothetical
Nonfibrotic MASH (F0 to F1)	Candidate SASP pro-inflammatory phase; candidate optimal window before fibrosis establishment	Pathogenic senescent hepatocytes; pro-inflammatory Kupffer cells	SHGS enrichment; FIB-4 as a fibrosis marker, not a senescence marker; elevated circulating IL-6 and IL-1β as exploratory markers	Short-course senolytic D+Q plus SASP modulation as a candidate trial strategy	Potentially optimal benefit-risk ratio, not validated	Hypothetical or early clinical association
Fibrotic MASH (F2 to F3)	Needs individualised definition based on fibrosis progression rate	Pro-fibrotic persistent senescent HSCs	FIB-4 and MRE as fibrosis markers, not senescence markers; elevated PRO-C3 as a candidate fibrogenesis marker	Senolytic plus anti-fibrotic combination as a candidate trial strategy	Requires careful evaluation	Hypothetical or early clinical association
Cirrhosis (F4) or HCC high risk	Markedly narrowed; careful evaluation after stringent screening	Individualised assessment; active HCC or high-risk lesions excluded from early trials	Child-Pugh as a hepatic function marker; platelet count as a safety parameter; HCC excluded by imaging	Routine clearance not proposed; individualised intervention after MDT discussion only in selected research settings	Risk usually outweighs benefit; active HCC is an exclusion criterion for early trials, not an absolute clinical contraindication	Hypothetical or clinical caution

All therapeutic window parameters and intervention strategies in this table are proposed for future testing and have not been validated in clinical trials.

2.3 Sequential Treatment Hypothesis

This testable sequential treatment hypothesis comprises three consecutive phases, with phase transitions guided by biomarker monitoring and decision-making. The induction phase lasts a candidate duration of 1 to 4 weeks, using an intermittent dosing regimen with senolytic agents to rapidly eliminate accumulated pathogenic senescent cells. This design is inspired by the ongoing TRUTH trial (identifier: NCT05056488), which employs dasatinib plus quercetin (D + Q) in an intermittent regimen of 3 consecutive days per week for 3 weeks, followed by a 4-week washout period, with this cycle repeated three times for a total of 21 weeks. The TRUTH study population, primary and secondary endpoints, and exclusion criteria are described in the primary trial registry record. However, no published protocol separate from the registry record was identified at the time of writing. Other senolytic clinical trials have also adopted a similar "short cycle, intermittent" approach, which can serve as a design reference [21,22]. The theoretical goal of the induction phase is to reduce local senescent cell burden in the liver, decrease sustained release of SASP-related inflammatory factors, and create favorable conditions for subsequent tissue repair. This phase is expected to disrupt the SASP-driven vicious cycle of inflammation and fibrosis. Intermittent dosing is preferred over continuous dosing for three reasons: allowing tissue repair and cell regeneration after senescent cell clearance, avoiding cumulative off-target toxicity from continuous exposure, and permitting observation of biomarker responses during washout periods. The optimal duration and drug selection for the induction phase still require dose-finding studies.

The maintenance phase lasts a candidate duration of 6 months or longer. It transitions to senomorphic agents combined with lifestyle intervention. Unlike senolytics, senomorphics modulate the phenotype and secretory activity of senescent cells without inducing cell death, mainly by suppressing the SASP [23]. The mammalian target of rapamycin inhibitor rapamycin and the AMPK activator metformin are relatively well-studied senomorphic agents. Rapamycin attenuates SASP signaling through mTOR inhibition. Metformin reduces SASP production by activating AMPK and suppressing nuclear factor-kappa B-dependent inflammatory signaling [24]. The core logic of the maintenance phase is that senescent cell generation is ongoing. Without fundamental correction of metabolic derangement, a single clearance episode cannot achieve durable benefit. Senomorphic intervention is needed to reduce the pathogenic potential of newly generated senescent cells, while lifestyle intervention addresses upstream senescence-inducing stimuli. The maintenance phase is expected to sustain the senescence remission state and prolong the therapeutic benefit of initial clearance.

The re-assessment phase uses dynamic biomarker monitoring to determine when senescent burden again exceeds a candidate pre-specified threshold. At that point, a repeat short-course clearance regime could be considered within a trial protocol. This phase embodies the dynamic adaptability of the treatment strategy. Key questions are what degree of senescent burden rebound warrants a new clearance round and whether cumulative safety of repeated clearance is acceptable. Evidence-based thresholds for re-treatment decisions are lacking, and this is a critical issue for future research. The sequential strategy has three theoretical advantages: avoiding cumulative toxicity from prolonged continuous exposure to senolytics, preserving physiologically functional senescent cells that participate in tissue repair, particularly transient adaptive senescence responses after acute injury, and consolidating the therapeutic effect (Fig. 2).

3. Biomarker Stratification Framework

Previous studies employed senescence markers as single detection indices. The core contribution of this study is to classify biomarkers into three categories according to clinical decision functions. A further contribution is an explicit evidence hierarchy that distinguishes preclinical, human observational, analytically validated, clinically validated, predictive, pharmacodynamic, and exploratory biomarkers. Different clinical decisions require biomarkers with distinct performance characteristics. Matching biomarker uses to analytical performance and clinical validation status helps avoid overinterpreting mechanistic research markers as clinical decision tools.

3.1 Patient Selection Biomarkers

Patient selection biomarkers are used before treatment initiation to identify individuals most likely to benefit from senolytic therapy. Their core evaluation criterion is the strength of association with senescent cell burden and SASP pathogenicity. Candidate biomarkers fall into three main categories. However, none of the currently available candidate markers has been validated as a predictive biomarker of senolytic response in MASLD.

The first category is immunohistochemical expression of p16 and p21 in liver tissue. p16 and p21 are cyclin-dependent kinase inhibitors that serve as core executors in DNA damage responses and oxidative stress-induced senescence [25]. Recent work developed a hepatocyte-specific senescence gene signature (SHGS) that outperforms existing models in predicting MASLD severity and progression. Its enrichment shows stepwise correlations with MASLD severity, MASH and HCC risk,

and clinical parameters including body mass index, aspartate aminotransferase, and FIB-4 [6]. SHGS provides an important tool for transcriptomics-based senescent burden assessment. Its superiority over traditional single markers lies in integrating expression information from multiple senescence-related pathways, thereby reflecting functional status more comprehensively than the mere presence of senescent cells [10]. However, SHGS is currently a prognostic and disease-association biomarker, not a validated predictive biomarker for selecting patients for senolytic therapy. Its association with disease severity does not establish that SHGS-high patients will respond to senolytics. Current clinical application of SHGS remains limited by the requirement for invasive liver biopsy to obtain transcriptomic data. This motivates research into alternative circulating biomarker strategies.

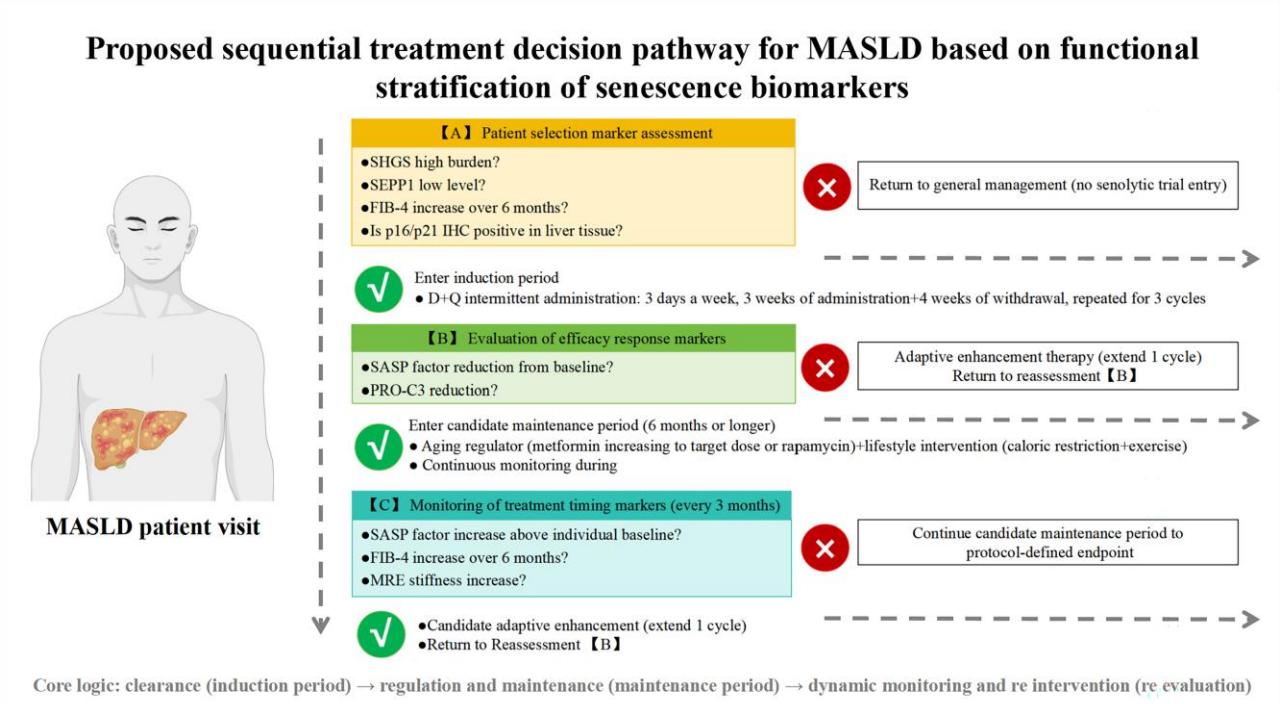


Figure 2. Proposed sequential treatment decision pathway for MASLD based on functional stratification of senescence biomarkers. This figure illustrates a candidate trial-design framework and is not a clinical algorithm. All decision nodes, biomarker assessments, numerical thresholds, phase durations, re-treatment rules, and stopping rules shown here are hypothetical research proposals for prospective testing and have not been validated in MASLD. Specific numerical thresholds are shown here only as illustrative examples of the types of criteria that future trials might test. They should not be used as clinical decision criteria. D + Q, dasatinib plus quercetin; MASLD, metabolic dysfunction-associated steatotic liver disease; MRE, magnetic resonance elastography; SASP, senescence-associated secretory phenotype; SHGS, Senescence-Hepatocyte Gene Signature; SEPP1, selenoprotein P.

The second category is a composite circulating SASP factor score, including interleukin-6, interleukin-1β, C-X-C motif chemokine ligand 10, and tumor necrosis factor-alpha [4]. The concentration of any single SASP factor in blood is subject to confounding from multiple non-hepatic sources. The composite score is designed to enhance specificity of the hepatic senescence burden signal by integrating multiple factors [26]. A limitation is that circulating SASP factors cannot distinguish their tissue of origin and may lack sensitivity when hepatic inflammation is relatively localized. These markers should therefore be described as exploratory and nonspecific, influenced by obesity, metabolic dysfunction, infection, and extrahepatic inflammation, rather than as direct hepatic senescence-specific selection tools.

The third category is low serum levels of selenoprotein P (SEPP1). SEPP1 is a selenium-containing antioxidant protein synthesized and secreted by the liver. A prospective case-control study enrolling 160 Egyptian participants showed that SEPP1 levels were significantly lower in MASLD patients than in healthy controls ($p < 0.001$), the lowest levels were observed in the significant fibrosis subgroup ($p = 0.025$ vs. non-significant fibrosis), SEPP1 correlated negatively with the homeostatic model assessment for insulin resistance ($r = -0.25$, $p = 0.001$) and body mass index ($r = -0.23$, $p = 0.004$) [27]. This suggests that SEPP1 may serve as a convenient biomarker for metabolic dysfunction and non-invasive fibrosis risk

stratification. SEPP1 has hepatic specificity and high analytical feasibility. However, its validation as a patient selection biomarker is less advanced than that of SHGS, and its independent predictive value needs confirmation in large, multicenter cohorts. SEPP1 should currently be regarded as an exploratory candidate marker, not as a validated senescence-specific patient-selection biomarker.

3.2 Treatment Timing Biomarkers

Treatment timing biomarkers determine the optimal time to initiate therapy and repeat treatment. Their core function is to identify patients in whose senescent burden is rising rapidly or SASP activity is intensifying. Candidate indicators can be examined from three aspects. First, a substantial increase in SASP factors above an individual's baseline may indicate a rise in senescence-related inflammatory burden. However, no validated numerical threshold currently exists for this purpose in MASLD, and direct evidence supporting a specific percentage increase is lacking [28]. Any such threshold should therefore be regarded as a candidate research question rather than a predefined criterion. Second, short-term disease progression detected by FIB-4 or magnetic resonance elastography (MRE) may serve as a candidate disease-progression endpoint rather than a senescence-specific biomarker. Specific numerical thresholds, such as a given absolute increase in FIB-4 or a given percentage increase in MRE stiffness over a defined interval, have not been validated in the context of senotherapy and should not be presented as established decision criteria [29]. The correlation between SHGS and FIB-4 provides additional rationale for its clinical application. Third, circulating senescence-associated extracellular vesicle microRNA profiles remain in early exploratory stages [30]. Their theoretical advantage is that extracellular vesicles may carry cell type-specific molecular information, potentially overcoming the limitation of traditional circulating markers in source tracing.

3.3 Response Biomarkers

Response biomarkers assess treatment efficacy and guide maintenance or discontinuation decisions. They need to detect changes attributable to intervention within a relatively short time after treatment initiation. Candidate indicators encompass three main directions. These are candidate pharmacodynamic or exploratory endpoints, not validated surrogate clinical endpoints.

One potential pharmacodynamic signal is a reduction in SASP factors after an initial treatment period, which may serve as indirect evidence of target engagement during the induction phase. However, the magnitude of reduction that should be considered meaningful, and the optimal time point for its assessment, remain unresolved [31]. The association between early SASP decline and long-term clinical benefit remains to be determined [32]. Although prior studies have reported that senolytic therapies can clear a proportion of senescent cells in preclinical and early clinical settings [33], evidence that senescent-cell clearance directly produces a corresponding reduction in circulating SASP factors in MASLD is lacking. We therefore do not propose a specific numerical SASP reduction threshold. Instead, any change in SASP factors should be treated as a candidate exploratory pharmacodynamic endpoint requiring prospective validation. Another is change in fibrogenesis activity reflected by the N-terminal propeptide of type III collagen (PRO-C3). PRO-C3 is a marker of active collagen synthesis. Compared with traditional fibrosis staging measures such as MRE or biopsy staging, it is more sensitive to dynamic changes in fibrotic activity [34]. A third candidate endpoint is change in MRE stiffness values at 6 months, providing non-invasive imaging evidence of fibrosis regression or absence of progression. A specific numerical reduction in MRE stiffness has been discussed as a possible early response signal based on MRE measurement variability [35], but direct clinical evidence supporting any particular threshold in the context of senotherapy is currently lacking. It therefore remains a candidate exploratory endpoint rather than an established response threshold. Combining short-term pharmacodynamic markers with medium-term tissue structural markers may provide a multi-level evidence framework for efficacy assessment.

3.4 Translational Pathway

The translational pathway for biomarkers is planned in three phases. Each phase corresponds to specific validation objectives and implementation strategies. The short-term goal is to establish a composite scoring system combining FIB-4 with circulating SASP factors and to conduct retrospective cohort studies validating its correlation with hepatic senescence markers. FIB-4 should be treated as a fibrosis or disease severity component, while circulating SASP factors are exploratory senescence-associated components. All data can be obtained from existing biobanks and clinical databases without additional prospective sampling. The medium-term goal is to validate SHGS performance in real-world patient cohorts and develop a serum-based SHGS equivalent (seSHGS) for minimally invasive patient stratification. The development pathway for seSHGS can be based on secreted proteins encoded by key genes in SHGS or their metabolites in blood. The long-term goal is to develop senescent cell-specific positron

emission tomography tracers for non-invasive visualization of systemic senescent cell burden. The technical challenge is the overlap of molecular markers between senescent cells and other cell states, which requires extensive optimization of tracer specificity (Table 2).

Table 2. Clinical decision function-based stratification of senescence biomarkers and translational pathway, with evidence hierarchy.

Functional category	Core decision question	Representative candidate indicators	Current validation stage	Evidence level	Translational milestones
Patient selection biomarkers	Who is most likely to benefit?	(1) SHGS by transcriptomics; (2) composite circulating SASP score including IL-6, IL-1β, CXCL10, and TNF-α; (3) low SEPP1	Preclinical to preliminary clinical validation	SHGS is prognostic or disease-association; SASP score and SEPP1 are exploratory	Short-term: establish FIB-4 plus circulating SASP composite score; conduct retrospective cohort validation without claiming predictive utility
Treatment timing biomarkers	When to initiate or repeat therapy?	(1) a substantial increase in SASP factors above an individual's baseline (no validated numerical threshold); (2) short-term FIB-4 increase over a defined interval (no validated numerical threshold); (3) MRE stiffness increase over a defined interval (no validated numerical threshold); (4) EV microRNA profiles, exploratory	First three have preliminary clinical associations but no validated thresholds; EV miRNA is early exploratory	Candidate thresholds; FIB-4 and MRE are disease progression markers, not senescence-specific	Medium-term: prospectively validate SHGS in multicentre cohorts; develop serum equivalent seSHGS
Response biomarkers	Is treatment effective? When to adjust?	(1) SASP factor reduction from baseline (magnitude and time point unresolved); (2) PRO-C3 reduction; (3) MRE stiffness reduction at 6 months (no validated numerical threshold)	Exploratory clinical evidence; TRUTH study protocol should be cited from the primary registry record	Candidate pharmacodynamic or exploratory endpoints	Long-term: develop senescence-specific PET tracers

Biomarker categories and evidence hierarchy are proposed for research purposes. No biomarker listed here has been validated as a clinical decision tool in MASLD senotherapy. Specific numerical thresholds shown in this table are illustrative examples only and should not be used as clinical decision criteria.

4. Safety Considerations

Clinical application of senolytic therapy requires attention to three core safety risks. The relative weight of these risks varies with disease stage and treatment phase and should be assessed individually. In the proposed trial framework, these risks should directly inform patient selection, dosing, re-treatment, and stopping rules. The following sections specify how each risk maps onto these four decision domains.

4.1 Off-Target Toxicity

Dasatinib inhibits platelet function. The risk is substantially elevated in patients with advanced liver disease complicated by thrombocytopenia [36]. Thrombocytopenia in cirrhotic patients involves hypersplenism, reduced thrombopoietin production, and bone marrow suppression. These patients are particularly susceptible to hematological toxicity from dasatinib. Navitoclax (ABT-263) and other B-cell lymphoma-2 family inhibitors cause severe hematological adverse events, including thrombocytopenia and neutropenia, limiting their tolerable dose range in liver disease [37]. Patients with chronic liver disease often develop consumptive thrombocytopenia due to hypersplenism and already have bleeding risks such as variceal hemorrhage. Off-target hematological toxicity is therefore particularly concerning [38]. Senolytics may also affect senescent cell clearance in extrahepatic tissues, producing systemic effects in the kidney, cardiovascular system, and skeletal muscle. These effects require systematic monitoring during long-term administration [39]. Mitigation

strategies include intermittent short-course regimens to allow normal tissue recovery during washout periods and exclusion of patients with Child-Pugh class B or C, because insufficient hepatic functional reserve reduces drug metabolic capacity and increases toxic exposure. In the proposed trial framework, these considerations support the following: (1) patient selection: excluding patients with severe thrombocytopenia or bleeding risk; (2) dosing: using intermittent rather than continuous dosing; (3) re-treatment: pre-specifying minimum intervals and cumulative dose limits; (4) stopping rules: pre-specifying hematological stopping rules. Child-Pugh B or C should be avoided in early trials.

4.2 Impaired Tissue Repair

Senescent cells serve transient physiological functions in wound healing and tissue remodeling after acute injury. This finding constrains the timing of senescence-clearing strategies. Premature application may interfere with adaptive transient senescence responses, delaying tissue repair or exacerbating injury [40]. In MASLD, this issue is particularly significant because hepatocyte regenerative capacity is a key determinant of disease prognosis. To mitigate this risk, intermittent dosing regimens are recommended. The rationale is to allow necessary adaptive senescence responses during washout periods, verify regenerative capacity after partial hepatectomy in preclinical studies, and exclude patients with recent planned surgery or active hepatic injury in clinical trials. These should be framed as proposed risk mitigation measures for future trials, including pre-specified exclusion criteria and regenerative capacity monitoring. In the proposed trial framework, these considerations translate into (1) patient selection: excluding patients with recent planned surgery or active hepatic injury; (2) dosing: using intermittent regimens; (3) re-treatment: verifying regenerative capacity before repeat clearance; (4) stopping rules: pre-specifying criteria for impaired regeneration.

4.3 The Dual Role in Tumorigenesis

Senescence has dual effects in tumor suppression and tumor promotion. This duality is the most profound safety challenge in applying senescence interventions to liver disease. Senescence exerts tumor-suppressive functions by preventing damaged cells from proliferating. Activation of the p53/p21 and p16/Rb pathways can permanently block cells harboring oncogenic mutations from entering the division cycle [41]. Inflammatory factors within the SASP can create a pro-tumorigenic microenvironment by promoting angiogenesis, epithelial-mesenchymal transition, and immune evasion, supporting growth of established tumors [42]. Gu et al. elucidated the molecular mechanism of the senescence paradox in MASH. Fructose-1,6-bisphosphatase 1 (FBP1) mediates hepatocyte senescence through regulation of the p53/p21 pathway, and loss of FBP1 relieves senescence suppression and promotes HCC formation [43].

The key insight is that in MASH, senescence itself may have a protective function in suppressing malignant transformation. Indiscriminate elimination of senescent hepatocytes may increase HCC risk under certain circumstances. This suggests that prioritizing clinical studies in patients with F2 to F3 fibrosis without high-grade dysplasia is more prudent. Strict risk-benefit assessment with exclusion of patients with active HCC is required in cirrhotic patients. However, the available evidence does not justify describing HCC as an absolute clinical contraindication. A more appropriate statement is that patients with active HCC or high-risk premalignant lesions should currently be excluded from early senolytic trials pending dedicated safety data. In the proposed framework, this translates into (1) patient selection: mandatory HCC screening and exclusion of active HCC or high-grade dysplastic nodules from early trials; (2) dosing: no modification specified; (3) re-treatment: long-term oncological surveillance before considering repeat clearance; (4) stopping rules: pre-specified oncological stopping rules and independent safety monitoring. From a broader perspective, tumor safety assessment of senotherapies should be embedded in trial stopping rules and independent safety monitoring.

5. Conclusions and Perspectives

Senotherapies in MASLD are advancing from mechanistic validation toward clinical translation. Successful translation will not occur automatically. It depends on precise patient stratification, optimized timing of treatment initiation, and rational sequential strategies. These elements form the necessary bridge from senescence mechanism research to precision senescence intervention. Emerging biomarkers such as SHGS and SEPP1 provide new tools for precision stratification. Ongoing clinical trials such as the TRUTH study are expected to provide pivotal first-in-human evidence on efficacy and safety of senolytics in fibrotic MASLD. Their results are not yet available, and the present framework should be revisited once they are reported. The sequential model of short-term senolytic clearance combined with long-term senomorphic modulation and metabolic maintenance offers a testable translational hypothesis. Its proposed core logic is to respect the heterogeneous roles of senescence across disease stages and to adapt to this heterogeneity through dynamic monitoring and stage transitions.

Validation of this hypothesis requires coordinated progression at multiple levels: mechanistic confirmation in stage-specific preclinical models, stepwise biomarker development from tissue to fluid compartments, and biomarker-driven adaptive clinical trial designs. The central question should be how to test stage-specific senotherapy in prospective studies, not how clinicians should currently treat patients. The field should shift focus from whether it works to who benefits, when, and how it can be implemented safely and effectively. This shift will determine whether senotherapies gain a substantial position in clinical management of MASLD rather than remaining confined to academic discussions of mechanisms. Future key milestones include prospective validation of SHGS and seSHGS in independent cohorts, completion of the first proof-of-concept trial of the sequential strategy, and determination of preclinical candidate molecules for senescence-specific imaging tracers. Achievement of these milestones would determine whether the framework has sufficient evidence for future clinical translation. It would not by itself establish evidence-based practice. The conclusion should therefore remain cautious and hypothesis-oriented.

Box 1. Definitions: Senolytic: an agent that selectively induces death of senescent cells.

Senomorphic: an agent that modulates the senescent phenotype or SASP without necessarily killing senescent cells.

Senescence burden: the abundance and pathogenic activity of senescent cells in a tissue.

Target engagement: evidence that a drug interacts with its intended molecular target.

Response biomarker: a biomarker that changes with treatment and may reflect pharmacodynamic effect, but requires validation as a surrogate of clinical benefit.

Author Contributions

Yunhang Chu led the conceptualization and writing of the original draft. Wenqi Jin and Weigeng She contributed to literature synthesis and figure preparation. Liwei Sun and Yan Leng provided critical intellectual input and assisted with data interpretation. Qi Meng supervised the project, provided critical intellectual input, and revised the final manuscript. All authors reviewed and approved the final manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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