THE ZOMBIE CELL ELIMINATION CHECKLIST

A Daily Protocol for Cellular Renewal & Senolytic Optimization

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Document Version: 2.4 | **Clinical & Translational Review Edition**
Focus: Targeted Clearance of Cellular Senescence, SASP Suppression, Autophagic Induction, and Mitochondrial Rejuvenation
Evidence Scope: Human Clinical Trials, Preclinical In Vivo Models, and Mechanistic Translational Data

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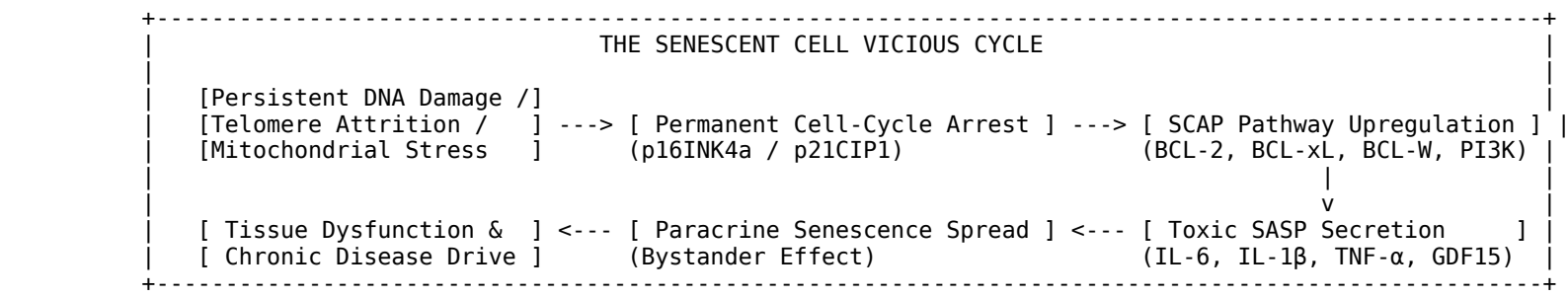
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1. EXECUTIVE SUMMARY: THE SENESCENT "ZOMBIE" CELL PARADIGM

Cellular senescence is a fundamental hallmark of aging characterized by a permanent arrest of cell division, resistance to programmed cell death (apoptosis), and the hyper-secretion of a toxic molecular cocktail termed the **Senescence-Associated Secretory Phenotype (SASP)**. Colloquially termed **"zombie cells,"** these metabolically active but non-replicating cells accumulate across tissues as organisms age, driven by persistent genomic damage, telomere attrition, oxidative stress, oncogenic activation, and mitochondrial dysfunction [PMC 6796530, Nature 2026].

While acute senescence serves crucial physiological functions—such as preventing the malignant transformation of damaged cells and orchestrating early wound healing—the chronic, unabated accumulation of senescent cells exerts catastrophic systemic effects. Senescent cells survive by upregulating **Senescent Cell Anti-Apoptotic**

Pathways (SCAPs), evading immune clearance, and converting healthy neighboring cells into senescent entities via paracrine SASP signaling [PMC 6197652, Lancet eBioMedicine 2025].



Why Eliminating Zombie Cells Matters

Preclinical investigations demonstrate that transplanting even small quantities of senescent cells into young, healthy animal models causes physical dysfunction, accelerates frailty, and induces premature multi-organ pathology [PMC 6796530]. Conversely, selectively clearing senescent cells—or neutralizing their secretory profile—yields profound therapeutic dividends:

- **Extended Healthspan & Lifespan:** Genetic or pharmacological clearance of senescent cells extends median lifespan in murine models and delays age-related physical and cognitive decline.
- **Restoration of Tissue Homeostasis:** Clearance restores muscle stem cell regenerative capacity, mitigates fibrotic remodeling in pulmonary and renal tissues, and improves vascular elasticity.
- **Suppression of Systemic Inflammaging:** Eliminating senescent cell burden drastically decreases circulating levels of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs).
- **Improved Physical Function:** Human pilot trials of senolytic therapy report measurable gains in physical performance metrics such as 6-minute walk distance [PMC 6796530, Lancet eBioMedicine 2025].

The Dual-Pronged Intervention Strategy

Targeting cellular senescence requires a coordinated, dual-pronged approach combining lifestyle, nutritional, and pharmacologic modalities:

1. **Senolytics:** Agents that transiently disrupt pro-survival SCAP networks, selectively triggering apoptosis in senescent cells while sparing healthy tissue. Due to their targeted mechanism, senolytics are administered in an **intermittent "pulse" (hit-and-run)** fashion.
2. **Senomorphics:** Compounds and lifestyle interventions (e.g., autophagy induction, exercise, time-restricted feeding, polyphenols) that suppress SASP expression, attenuate NF-κB/mTOR hyperactivation, and enhance immune-mediated phagocytosis on a **continuous daily basis**.

This protocol integrates translational gerontology into an actionable daily, weekly, and monthly regimen designed to minimize senescent cell accumulation, extinguish SASP-driven inflammaging, and promote cellular renewal.

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2. THE MOLECULAR BIOLOGY OF CELLULAR SENESCENCE & THE SASP

2.1 Drivers of Senescence: DNA Damage, Telomeres, and Oncogenes

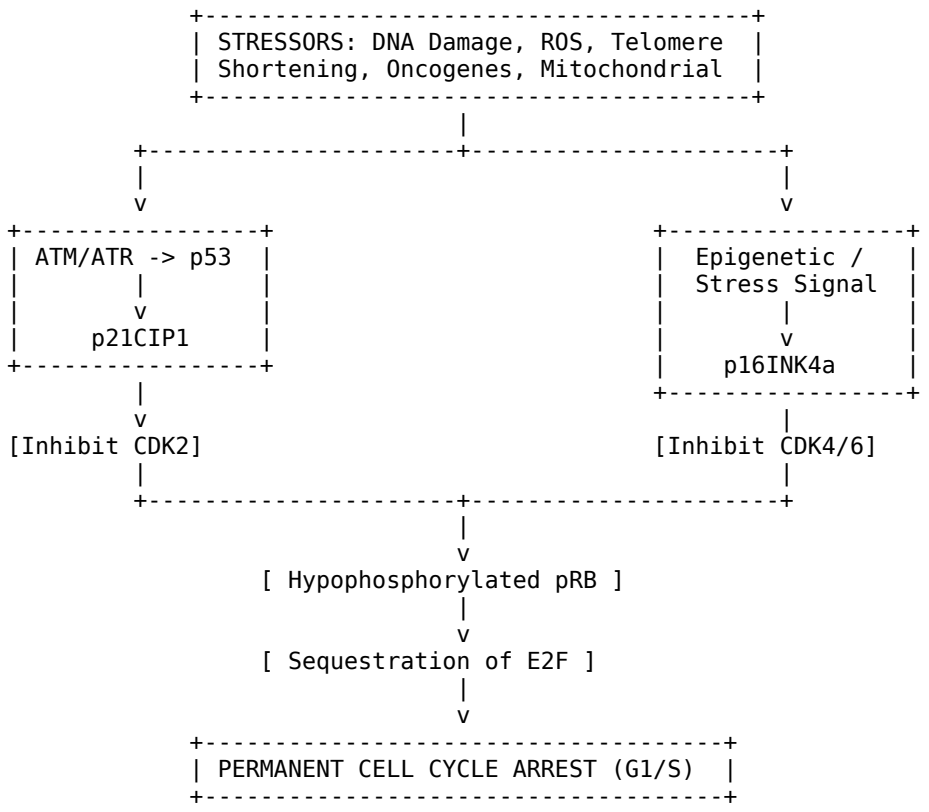
Cellular senescence occurs when a normal cell encounters persistent, irreparable cellular stress. The principal initiating triggers include:

1. **Replicative Senescence (Telomere Depletion):** With each round of cell division, telomeres undergo progressive shortening due to the end-replication problem. When telomeres reach a critical minimum threshold (the Hayflick limit), uncapped chromosome ends trigger a chronic **DNA Damage Response (DDR)** mediated by ATM/ATR kinases and downstream CHK1/CHK2 signaling.
2. **Stress-Induced Premature Senescence (SIPS):** Extrinsic stressors—including excessive reactive oxygen species (ROS), ultraviolet radiation, chemotherapeutic agents, lipid peroxidation products (e.g., 4-HNE), and chronic hyperglycemia—induce complex double-strand breaks (DSBs) and oxidative base modifications in non-telomeric genomic DNA.
3. **Oncogene-Induced Senescence (OIS):** Mutational activation of proto-oncogenes (e.g., *BRAF* V600E, *RAS*) or the loss of tumor suppressors (e.g., *PTEN*) causes replication stress and hyper-mitogenic arrest, acting as a critical autonomous defense against tumorigenesis.

4. **Mitochondrial Dysfunction-Associated Senescence (MiDAS):** Severe mitochondrial electron transport chain defects, loss of mitochondrial membrane potential, and altered NAD+/NADH ratios trigger senescence with a distinct, low-IL-1 SASP profile.

These pathways converge on two central, mutually reinforcing cell-cycle arrest axes:

- **The p53–p21CIP1 (CDKN1A) Pathway:** Acute DDR triggers ATM/CHK2-mediated phosphorylation and stabilization of p53, which transcriptionally upregulates p21CIP1, inhibiting Cyclin E-CDK2 complexes and enforcing G1 cell-cycle arrest.
- **The p16INK4a (CDKN2A)–Retinoblastoma (pRB) Pathway:** Epigenetic derepression of the *INK4a/ARF* locus induces p16INK4a accumulation, which binds CDK4/6, preventing CDK4/6-mediated phosphorylation of pRB. Hypophosphorylated pRB remains stably bound to E2F transcription factors, permanently silencing genes required for S-phase entry [PMC 12181168, PMC 9970302].



The Tissue-Specific Burden of Senescence

Senescent cell accumulation is not uniform; it follows tissue-specific kinetics that determine the clinical expression of aging:

Tissue / Organ System	Dominant Senescent Cell Type	Clinical Consequence
Adipose Tissue	Adipocyte progenitors, preadipocytes	Metabolic dysfunction, insulin resistance, ectopic lipid deposition
Skeletal Muscle	Satellite cells, myofibers	Sarcopenia, reduced regenerative capacity, frailty
Skin (Dermis/Epidermis)	Fibroblasts, melanocytes, keratinocytes	Wrinkling, impaired barrier repair, hyperpigmentation, delayed wound closure
Vasculature	Endothelial cells, smooth muscle cells	Arterial stiffness, endothelial dysfunction, hypertension
Lung	Alveolar epithelial cells, fibroblasts	Idiopathic pulmonary fibrosis, reduced gas exchange
Kidney	Tubular epithelial cells	Fibrosis, declining glomerular filtration rate
Brain	Microglia, astrocytes, neurons	Neuroinflammation, cognitive decline, neurodegeneration
Bone	Osteocytes, osteoblast progenitors	Osteoporosis, impaired fracture healing

2.2 Pro-Survival Senescent Cell Anti-Apoptotic Pathways (SCAPs)

Under normal biological conditions, severe genomic damage triggers programmed apoptosis through internal or external death receptor cascades. Senescent cells, however, exhibit profound resistance to apoptosis.

To survive their own toxic intracellular environment, senescent cells upregulate specific anti-apoptotic survival nodes, collectively referred to as **SCAP networks**:

- **BCL-2 Protein Family Overexpression:** BCL-2, BCL-xL (*BCL2L1*), and BCL-W sequester pro-apoptotic BH3-only proteins (BIM, PUMA, NOXA, BID) and prevent BAX/BAK oligomerization on the outer mitochondrial membrane, blocking cytochrome c release.
- **PI3K / AKT / mTOR Signaling Axis:** Hyperactivation of AKT suppresses pro-apoptotic Bad and Forkhead transcription factors (FOXO) while stabilizing cell survival.
- **Ephrin / Eph Receptor Kinases:** Eph receptors (specifically EphA2/B4) phosphorylate survival proteins and inhibit death receptors.
- **Hsp90 Chaperone Networks:** Hsp90 stabilizes mutated or misfolded pro-survival client kinases (e.g., AKT, CDK4, RIPK1).
- **p21–Serpine-1 Axis & MDM2 Regulators:** Downstream survival nodes that insulate senescent cells from intrinsic apoptotic triggers.

Senolytic pharmacology relies upon the temporary inhibition of these specific SCAP nodes. Because senescent cells are metabolically strained and burdened with high pro-apoptotic signals held in check only by SCAPs, transiently knocking down SCAP proteins causes senescent cells to rapidly collapse into apoptosis, while normal, unstressed cells tolerate the temporary inhibition without loss of viability [PMC 6796530, PMC 6197652].

The Critical Concept — The Apoptotic Threshold: Senescent cells exist in a state of "apoptotic priming." Their baseline pro-apoptotic load (BAX, BAK, BIM, PUMA) sits perilously close to the mitochondrial outer membrane permeabilization (MOMP) threshold. A healthy cell maintains a wide margin between its pro- and anti-apoptotic balance; a senescent cell maintains a razor-thin margin. Thus, a brief, partial inhibition of SCAPs —insufficient to push a healthy cell over the edge—is sufficient to tip a senescent cell into irreversible MOMP and death.

2.3 The Senescence-Associated Secretory Phenotype (SASP) Dynamics

The defining feature of senescent cells that drives tissue destruction is the SASP. Senescent cells become miniature factories producing dozens of bioactive proteins:

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|                              THE SASP MOLECULAR ARSENAL                                   |
|                                                                                           |
|  PRO-INFLAMMATORY CYTOKINES:       IL-6, IL-1α, IL-1β, IL-8, TNF-α                        |
|  CHEMOKINES:                       MCP-1 (CCL2), MIP-1α (CCL3), GRO-α (CXCL1)             |
|  MATRIX METALLOPROTEINASES:        MMP-1, MMP-3, MMP-9, MMP-12 (Destroys Extracellular Matrix)  |
|  GROWTH & DIFFERENTIATION FACTORS: GDF15, TGF-β, VEGF, Amphiregulin                       |
|  PRO-HEMOSTATIC / FIBROTIC:        PAI-1 (Serpine-1), IGFBP-3, IGFBP-7                    |
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Upstream Regulation of the SASP

The transcriptional synthesis of SASP factors is governed by several master molecular hubs:

1. **NF-κB (Nuclear Factor kappa B):** Chronic ATM-dependent DDR signaling triggers continuous phosphorylation and nuclear translocation of the p65/p50 NF-κB heterodimer.
2. **cGAS-STING Pathway:** Senescent cells frequently experience nuclear envelope breakdown and cytoplasmic chromatin fragment (CCF) leakage or mitochondrial DNA (mtDNA) release. Cytosolic cyclic GMP-AMP synthase (cGAS) binds this free double-stranded DNA, synthesizing cGAMP and activating Stimulator of Interferon Genes (STING), driving vigorous NF-κB and IRF3 activation.
3. **mTORC1 (Mechanistic Target of Rapamycin Complex 1):** Hyperactive mTORC1 selectively accelerates the translation of membrane-bound IL-1α, which acts in an autocrine loop through IL-1R to amplify NF-κB activation, while simultaneously phosphorylating 4E-BP1 and ZFP36L1 to stabilize SASP mRNAs.
4. **C/EBPβ & p38 MAPK:** Cooperate with NF-κB to sustain chronic cytokine production.

Paracrine Senescence Transmission ("The Bystander Effect")

SASP is not contained within the parent senescent cell. Secreted factors—notably IL-1β, IL-6, TGF-β, and extracellular vesicles (EVs) loaded with microRNAs—bind receptors on adjacent healthy somatic cells, generating elevated intracellular ROS, initiating DNA damage, and forcing neighboring cells into secondary senescence. This creates a self-amplifying cascade that degrades extracellular matrix integrity, induces stem cell exhaustion, triggers microvascular rarefaction, and establishes a chronic pro-thrombotic and pro-inflammatory microenvironment.

The clinical consequence is a self-reinforcing loop: each newly recruited senescent cell adds to the SASP burden, which in turn recruits more cells. This is why intervention must address **both** the existing senescent cell

population (senolytic clearance) **and** the signaling environment that continues to recruit new ones (senomorphic suppression).

2.4 Senolytics vs. Senomorphics: Elimination vs. Suppression

Understanding the distinction between senolytics and senomorphics is critical for structuring a safe, effective protocol:

Attribute	Senolytic Modality	Senomorphic Modality
Primary Mechanism	Selectively kills senescent cells by inhibiting SCAP anti-apoptotic nodes (BCL-2/xL, PI3K/AKT).	Inhibits SASP secretion and NF-κB/mTOR activity without killing the cell.
Dosing Paradigm	**Intermittent Pulse ("Hit-and-Run"):** 2–3 consecutive days once monthly or quarterly.	**Continuous Daily Administration:** Daily lifestyle habits, nutrition, and supplements.
Cellular Fate	Permanent apoptotic clearance followed by macrophage phagocytosis.	Cell remains alive but metabolically "silenced" and non-inflammatory.
Key Compounds	High-dose Fisetin, Quercetin, Dasatinib, Navitoclax (ABT-263).	Curcumin, Resveratrol, Spermidine, NMN/NR, Metformin, Rapamycin, Vitamin D.
Therapeutic Advantage	Complete elimination; does not require continuous drug exposure or chronic receptor blockade.	Prevents paracrine spread; suppresses systemic inflammation on a day-to-day basis.
Clinical Risk Profile	Potential temporary immune/platelet shifts; requires strict recovery intervals.	Risk of chronic pathway inhibition (e.g., continuous mTOR inhibition blunting muscle protein synthesis).

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3. THE COMPREHENSIVE DAILY PROTOCOL (TIME-BLOCK CHECKLIST)

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|                                DAILY CELLULAR RENEWAL TIMELINE                                |
|                                                                                               |
|   [06:00 - 08:00]  Morning Photobiology, Hydration, Autophagic Priming                        |
|   [08:00 - 11:30]  Extended Morning Fasting Zone & Deep Work                                  |
|   [11:30 - 12:30]  Meal 1: High-Polyphenol, Protein-Rich Break-Fast + Daily Base Stack        |
|   [14:00 - 16:30]  Resistance Training / HIIT Session                                         |
|   [16:30 - 17:30]  Meal 2 / Feeding Window Close + Post-Workout Recovery                      |
|   [18:00 - 21:00]  Autophagic Fasting Transition, Circadian Wind-Down, Stress Mitigation      |
|   [21:00 - 22:30]  Pre-Sleep Stack, Magnesium, Thermoregulation, Sleep Architecture           |
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3.1 Phase I: Morning Protocol (06:00 – 11:30)

1. Circadian Photobiology & Suprachiasmatic Alignment

- **Natural Sunlight Exposure:** Within 30–60 minutes of waking, expose eyes and skin to direct outdoor sunlight for **15–30 minutes** (minimum 10,000 lux; 30–45 minutes on overcast days). Do not wear sunglasses.
- **Molecular Mechanism:** Retinal ganglion cells containing melanopsin transmit photon signals directly to the suprachiasmatic nucleus (SCN). This suppresses daytime melatonin production, activates cortisol awakening response (CAR), and synchronizes central and peripheral cellular clocks (BMAL1/CLOCK and PER/CRY complexes). Proper circadian alignment optimizes nocturnal autophagic clearance and prevents circadian desynchrony-induced SASP upregulation.

2. Morning Hydration, Electrolytes & Autophagic Priming

- **Electrolyte Hydration Formula:**
  - 500–750 mL filtered water upon waking.
  - 1/4 tsp Himalayan pink salt or sea salt (~500 mg elemental sodium).
  - 100–200 mg potassium chloride/citrate.
  - Freshly squeezed lemon juice (provides bioflavonoids and citrate).
- **Autophagic Priming Beverage (Optional):**
  - High-EGCG Organic Green Tea (Matcha or Sencha) OR single-origin black coffee (consumed black, zero calories/sweeteners).

- *Mechanism:* EGCG (epigallocatechin gallate) and caffeine stimulate hepatic and systemic AMPK phosphorylation while inhibiting basal mTORC1, prolonging nocturnal macroautophagy and mitophagy.

3. Daily Maintenance Senomorphic & Senolytic Base Stack

Take with morning hydration or at the end of the morning fast:

- **NAD+ Precursor: NMN (Nicotinamide Mononucleotide) 500–1,000 mg** OR **NR (Nicotinamide Riboside) 300–600 mg** (sublingual or oral).
  - *Mechanism:* Elevates intracellular NAD+ pools, stimulating Sirtuin 1 and Sirtuin 3 (SIRT1/SIRT3) deacetylase activity, promoting DNA repair, suppressing NF-κB transcription, and maintaining mitochondrial quality control.
- **Spermidine (Wheat Germ Extract or Trihydrochloride): 1.2 mg to 3.0 mg daily** [PMC 5807086].
  - *Mechanism:* Promotes cellular autophagy and translation factor eIF5A hypusination; demonstrated to be safe and well-tolerated in older adults [PMC 5807086].
- **Trans-Resveratrol: 250–500 mg daily** (micronized or taken with a small amount of healthy lipid for bioavailability).
  - *Mechanism:* Allosteric SIRT1 activator; downregulates p38 MAPK phosphorylation and dampens SASP expression.
- **Bioavailable Curcumin (BCM-95, Meriva Phytosome, or with Piperine): 500–1,000 mg daily**.
  - *Mechanism:* Senomorphic master regulator; suppresses IκB kinase (IKK) phosphorylation, blocking NF-κB nuclear translocation and suppressing IL-6, IL-1β, and MMP transcription.

3.2 Phase II: Afternoon Protocol (11:30 – 17:30)

1. Time-Restricted Feeding (TRF) & Nutrient Timing

- **Feeding Window Strategy:** Implement a strict **16:8 or 18:6 Time-Restricted Feeding schedule** (e.g., 11:30 AM to 05:30 PM or 12:00 PM to 08:00 PM) [PMC 10940141, MDPI Nutrients 2026].
- **Physiological Rationale:** Prolonged daily nutrient depletion (16+ hours) lowers circulating insulin and IGF-1, de-represses transcription factor EB (TFEB) and FOXO3a, and activates AMPK-mediated autophagic flux. Human clinical trials confirm that 16:8 TRF significantly reduces circulating SASP markers, systemic TNF-α, and lipid peroxidation while preserving lean mass [PMC 10940141].

2. Polyphenol-Dense "Zombie-Clearing" Nutrition

Every meal during the feeding window must be deliberately constructed with high-potency senolytic and senomorphic whole foods:

SENOLYTIC & SENOMORPHIC WHOLE FOOD MATRIX

ACTIVE COMPONENT	PRIMARY FOOD SOURCES	TARGET MOLECULAR ACTION
Fisetin	Strawberries (160 mg/kg), Apples, Persimmons, Onions, Cucumbers	BCL-2/xL down-regulation; SCAP pathway disruption
Quercetin	Red Onions, Capers, Kale, Cranberries, Buckwheat, Organic Apple Skins	PI3K/AKT inhibition; Hsp90 chaperone destabilization
EGCG & Catechins	Ceremonial Grade Matcha, Green Tea, Organic Black/Oolong Teas	AMPK activation; SASP transcriptional arrest
Anthocyanins	Wild Blueberries, Blackberries, Pomegranate, Dark Tart Cherries	FOXO3a induction; Microvascular ROS suppression
Sulforaphane	Broccoli Sprouts (3-day fresh), Cruciferous Vegetables (Mustard Seed)	Nrf2 phase II antioxidant pathway; p21 stabilization in healthy cells
Spermidine	Aged Parmesan Cheese, Natto, Shiitake Mushrooms, Green Peas, Wheat Germ	Autophagosome-lysosome fusion; eIF5A hypusination pathway
Oleocanthal & Hydroxytyrosol	Extra Virgin Olive Oil (Cold-Pressed, High Polyphenol >500 mg/kg)	COX-1/2 inhibition; NF-κB blockade

3. Targeted Exercise Physiology: Resistance Training & HIIT

Exercise is the most potent physiological stimulus for clearing senescent cells and purging SASP from skeletal muscle and systemic circulation.

- **Resistance Training (Hypertrophy & Strength Focus):**
  - **Frequency & Dose:** 3–4 sessions per week (45–60 minutes per session), targeting compound multi-joint movements (Squats, Deadlifts, Overhead Press, Pull-ups).
  - **Mechanisms & Evidence:** Clinical and translational trials reveal that progressive resistance training significantly reduces muscle tissue levels of **p16INK4a**, **p21CIP1**, and key SASP factors (IL-6, TNF-α, MMP-9) while rejuvenating satellite stem cell pools and improving metabolic insulin sensitivity [PMC 12181168, PMC 9970302].
- **High-Intensity Interval Training (HIIT) & Zone 2 Cardio:**
  - **HIIT:** 1–2 sessions per week (e.g., 4–6 rounds of 30 seconds all-out sprint / 90 seconds active recovery on an air bike, rower, or track).
  - **Zone 2 Aerobic Conditioning:** 120–150 minutes per week at blood lactate 1.5–2.0 mmol/L (conversational pace).
  - **Mechanisms:** HIIT triggers rapid acute metabolic stress, depleting cellular ATP and triggering massive AMPK phosphorylation, which upregulates mitochondrial biogenesis (PGC-1α) and drives autophagic clearance of senescent endothelial and immune cells. Zone 2 training optimizes mitochondrial fatty acid oxidation capacity and clears damaged cellular debris.

Exercise Sequencing Note: Schedule resistance training in the mid-afternoon after the first meal, when muscle protein synthesis sensitivity and glycogen availability are optimized. Avoid scheduling HIIT within 24 hours of a senolytic pulse day, as the acute inflammatory and repair demands of maximal exercise may compete with the immune clearance of apoptotic senescent cell debris.

3.3 Phase III: Evening & Pre-Sleep Protocol (17:30 – 22:30)

1. Feeding Window Closure & Fasting Transition

- Conclude the final meal at least **3 to 4 hours before bedtime** (ideal window closure: 17:30–18:30).
- *Mechanism:* Ceasing nutrient intake prior to evening prevents nocturnal hyperinsulinemia, maintains low core body temperature, permits nocturnal growth hormone pulsatility, and allows the gastrointestinal system and liver to enter the autophagic renewal phase undisturbed.

2. Evening Autophagy & Neuroendocrine Support Stack

Administer 60–90 minutes before sleep:

- **Vitamin D3 + K2 (MK-7): 2,000–5,000 IU D3 + 100–200 mcg K2**.
  - *Evidence:* Randomized controlled data demonstrated that **2,000 IU/day of Vitamin D3 significantly slowed telomere shortening** and modulated inflammatory senescence markers over longitudinal follow-up [Harvard Gazette 2025].
- **High-Absorption Magnesium Chelate (Glycinate / L-Threonate): 300–400 mg elemental magnesium**.
  - *Mechanism:* Essential cofactor for over 300 enzymatic reactions, including DNA repair enzymes (PARP1), telomerase stability, and NMDA receptor modulation. Enhances deep slow-wave sleep.
- **L-Theanine / Apigenin / Chamomile Extract: 200 mg L-Theanine + 50 mg Apigenin**.
  - *Mechanism:* Apigenin acts as a natural CD38 inhibitor (preserving intracellular NAD+ levels) and modulates GABA-A receptors to induce relaxation without altering sleep architecture.

3. Circadian Wind-Down, Temperature & Sleep Architecture

- **Blue-Light Mitigation:** After sunset, eliminate overhead fluorescent/LED lighting. Wear amber/red blue-blocking glasses or utilize incandescent/low-kelvin (<2200K) lighting.
- **Thermoregulation for Autophagic Sleep:** Maintain bedroom ambient temperature at **65°F to 68°F (18°C to 20°C)**.
  - *Mechanism:* A drop in core body temperature is an obligate signal for initiation of restorative Stage 3 Slow-Wave Sleep (SWS).
- **Sleep Quantity & Quality Target:** 7.5 to 9.0 hours of uninterrupted sleep.
  - *Mechanism:* Deep slow-wave sleep activates the brain's **glymphatic system**, clearing toxic protein aggregates (amyloid-beta, hyperphosphorylated tau) and senescent glial cell debris, while low nocturnal insulin allows systemic macroautophagy to peak.

4. Stress Management & Parasympathetic Activation

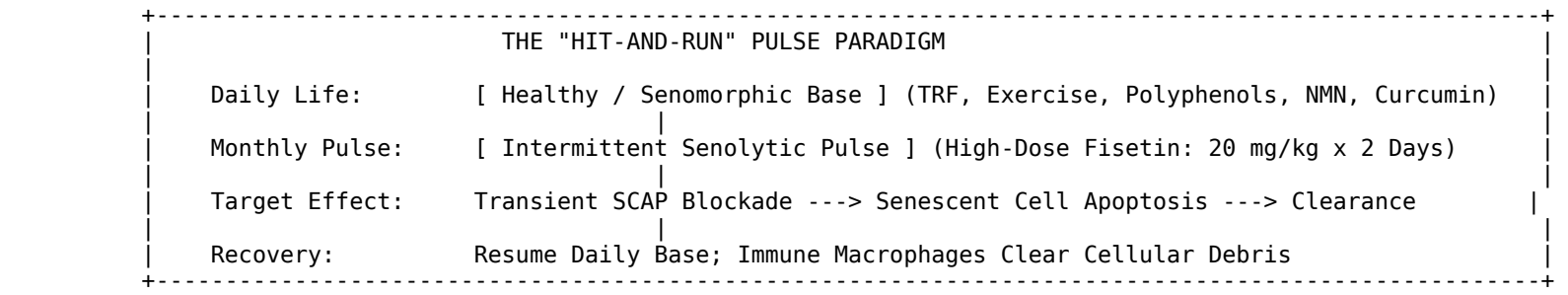
Chronic psychological stress elevates circulating cortisol, which directly upregulates NF-κB and amplifies SASP expression while accelerating telomere attrition in immune cells. Structured stress mitigation is therefore not optional—it is a core senomorphic intervention.

- **Slow Nasal Breathing (Resonance Frequency):** 5–6 breaths per minute for 10 minutes (e.g., 4-second inhale, 6-second exhale). Increases vagal tone and heart rate variability (HRV).

- **Box Breathing:** 4-count inhale, 4-count hold, 4-count exhale, 4-count hold — 5 minutes.
- **Mindfulness-Based Stress Reduction (MBSR):** 10–20 minutes of guided body-scan meditation.
- **Gratitude / Journaling:** 5 minutes of written reflection to reduce rumination-driven HPA axis activation.
- **Nature Exposure:** A 20–30 minute evening walk in green space, which lowers salivary cortisol and blood pressure.

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4. WEEKLY, MONTHLY, AND INTERMITTENT SENOLYTIC "PULSE" PROTOCOLS



4.1 The Hit-and-Run Intermittent Dosing Rationale

Unlike conventional pharmaceuticals that require continuous blood levels to sustain receptor blockade or enzyme inhibition, **senolytics operate via an intermittent "hit-and-run" mechanism**:

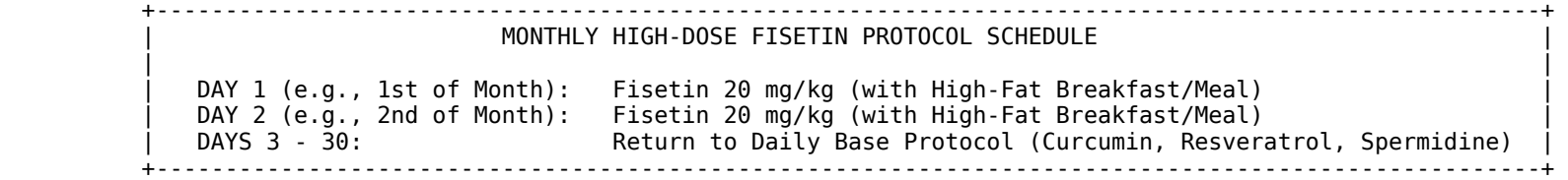
1. **Biological Turnover:** Senescent cells take weeks to months to develop and re-accumulate following stressful insults.
2. **Selective Apoptotic Trigger:** A brief 24- to 72-hour pulse of a senolytic compound is sufficient to disable SCAP networks (e.g., BCL-2, BCL-xL, PI3K), triggering an irreversible apoptotic cascade in primed senescent cells [PMC 6796530, PMC 6197652].
3. **Safety & Toxicity Avoidance:** Continuous administration of senolytics is counterproductive and potentially dangerous; chronic BCL-2/xL inhibition can cause thrombocytopenia, neutropenia, impaired tissue repair, and delayed wound healing.
4. **Immune Clearance Window:** The interval between pulses allows macrophages and natural killer cells to phagocytose apoptotic debris without overwhelming the reticuloendothelial system.

4.2 High-Dose Fisetin Monthly Pulse Protocol

Fisetin, a naturally occurring flavonol found in strawberries and apples, was identified in comprehensive screening as among the most potent natural senolytic agents, selectively reducing senescent cell markers across multiple human cell types and murine tissues [PMC 6197652].

The Intermittent Dosing Protocol

- **Target Dosage: ~20 mg per kilogram of body weight per day** (equivalent to approximately **1,400 mg to 1,800 mg/day** for a 70–90 kg individual) [Fishtown Medicine, PMC 6197652].
- **Administration Schedule:** Taken for **2 consecutive days per month** (e.g., the first Saturday and Sunday of each month). Repeat monthly for 3 to 6 consecutive cycles, followed by a clinical reassessment period.
- **Bioavailability Strategy:** Fisetin has poor aqueous solubility. It must be administered with a high-fat meal (e.g., extra virgin olive oil, avocado, or yogurt) or formulated in a lipid/liposomal/phytosomal matrix to ensure therapeutic plasma concentrations.



4.3 Quercetin Intermittent Pulse Protocol

Quercetin, a bioflavonoid abundant in red onions and capers, acts primarily through the inhibition of the PI3K/AKT and serpine-1 SCAP axes [PMC 6796530].

- **Pulse Dosing: 1,000 mg to 1,250 mg daily** for **2 to 3 consecutive days**, administered once monthly or bi-monthly.
- **Synergistic Co-Factors:** Frequently paired with Vitamin C (500 mg) and Bromelain (200 mg) to prevent flavonoid oxidation and enhance intestinal absorption.

4.4 Clinical-Grade Intermittent Senolytic Protocols (Dasatinib + Quercetin)

The combination of **Dasatinib (D)**—a tyrosine kinase inhibitor used in oncology—and **Quercetin (Q)** represents the most thoroughly validated senolytic cocktail in translational medicine, providing complementary SCAP inhibition: Dasatinib inhibits the EphA2/tyrosine kinase survival nodes (clearing senescent adipocyte and endothelial progenitors), while Quercetin inhibits BCL-xL and PI3K/AKT nodes (clearing senescent endothelial and mesenchymal cells) [PMC 6796530, Nature 2026, Lancet eBioMedicine 2025].

Clinical Trial Evidence & Dosage Parameters

- **Validated Regimen: Dasatinib 100 mg orally + Quercetin 1,000–1,250 mg orally per day** for **2 to 3 consecutive days** per cycle [PMC 6796530, Lancet eBioMedicine 2025].
- **Cycle Frequency:** Administered every 14 to 28 days for 2–3 cycles in human clinical trials (e.g., diabetic kidney disease, idiopathic pulmonary fibrosis, and Alzheimer's disease cohorts).
- **Observed Clinical Outcomes:** Trials demonstrated significant reduction in adipose and epidermal **p16INK4a** and **p21CIP1** expression, marked suppression of circulating SASP factors (IL-6, IL-1α, TNF-α, MMP-9, MMP-12), and improved physical performance metrics (6-minute walk distance) [PMC 6796530, Lancet eBioMedicine 2025].
- **Clinical Caveat:** *Dasatinib is a prescription pharmaceutical with potential adverse effects (fluid retention, pleural effusion, myelosuppression, QT prolongation) and must strictly be administered under physician supervision in a medical setting.*

4.5 Weekly Autophagic Fasting Windows & Rapamycin Considerations

1. Weekly 24-Hour Autophagic Fast

- **Protocol:** Implement a weekly **24-hour water/electrolyte fast** (e.g., dinner-to-dinner once weekly, such as Sunday 18:00 to Monday 18:00).
- **Mechanism:** Sustained liver glycogen depletion triggers deep autophagic degradation of damaged organelles (mitophagy, ribophagy) and induces senescent cell metabolic stress, priming senescent cells for elimination.

2. Low-Dose Intermittent Rapamycin (Sirolimus) Dynamics

- **Mechanistic Profile:** Rapamycin is an allosteric inhibitor of mTORC1. At low, intermittent doses, it acts as a premier **senomorphic agent**, potently silencing SASP translation and restoring stem cell function.
- **Intermittent Dosing Schedule: 5 mg to 10 mg once weekly** (pulsed single dose to allow mTORC2 recovery and prevent continuous immunosuppression) [PMC 13082878].
- **The Exercise-Rapamycin Interference Caveat:** *Because mTORC1 activation is required for post-exercise muscle protein synthesis and satellite cell accretion, rapamycin must NOT be taken within 24–48 hours of heavy resistance training. Schedule the weekly rapamycin pulse on a scheduled complete rest day* [PMC 13082878].

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5. EVIDENCE SUMMARY & MASTER FORMULATION MATRIX

5.1 Evidence Grading Framework

To ensure scientific rigor, all compounds and interventions in this protocol are categorized according to evidence hierarchy:

- **Level I (Human RCT / Interventional Data):** Replicated human clinical trials demonstrating statistically significant reductions in senescence biomarkers, SASP, or functional endpoints.
- **Level II (Preclinical In Vivo Models):** Peer-reviewed rodent/mammalian studies demonstrating senescent cell clearance, lifespan extension, or organ regeneration.

- **Level III (Mechanistic / In Vitro Data):** Cell culture assays demonstrating SCAP inhibition, apoptosis induction, or NF-κB/mTOR modulation.

5.2 Master Evidence Table: Senolytic & Senomorphic Compounds

Compound / Modality	Target SCAP / Pathway	Classification	Evidence Level	Clinical / Standard Dose	Primary Human & Preclinical Findings	Key Scientific Citations
Dasatinib + Quercetin (D+Q)	EphA2, BCL-xL, PI3K / AKT	Senolytic (Synthetic + Natural)	**Level I** (Human Trials)	100 mg D + 1,000–1,250 mg Q (2–3 days/pulse)	Clears senescent cells in human adipose & skin; reduces IL-6, TNF-α, MMPs; improves 6-min walk distance in IPF.	PMC 6796530, Nature 2026, Lancet eBioMedicine 2025
Fisetin	BCL-2, BCL-xL, PI3K / AKT, NF-κB	Senolytic & Senomorphic	**Level I / II** (Human Trials ongoing, robust In Vivo)	~20 mg/kg/day (2 consecutive days/month)	Potent natural senolytic in screening; restores tissue homeostasis, reduces oxidative stress and SASP markers.	Fishtown Medicine, PMC 6197652
Quercetin (Monotherapy)	PI3K / AKT, BCL-2 family, Serpine-1	Senolytic & Senomorphic	**Level II / III**	500–1,000 mg/day (Daily) or 1,250 mg (Pulse)	Suppresses inflammatory cytokines; clears senescent endothelial cells; antioxidant and anti-fibrotic.	PMC 6796530, PMC 6197652
Curcumin	NF-κB, IKK, STAT3, p38 MAPK	Senomorphic	**Level I / II**	500–1,000 mg/day (Bioavailable phytosome)	Master SASP silencer; blocks NF-κB transcription; reduces circulating CRP, IL-1β, and IL-6 in clinical cohorts.	PMC 6197652
Trans-Resveratrol	SIRT1 activation, NF-κB, p38 MAPK	Senomorphic	**Level I / II**	250–500 mg/day (Micronized)	Upregulates SIRT1 deacetylation; mitigates DNA damage-induced SASP; improves endothelial nitric oxide synthase (eNOS).	PMC 6197652
Spermidine	Autophagy (TFEB), eIF5A hypusination	Senomorphic & Autophagy Inducer	**Level I / II**	1.2–3.0 mg/day (Daily)	Induces macroautophagy; demonstrated safe and well-tolerated in older adults; improves memory scores.	PMC 5807086
NMN / NR	NAD+ pool, SIRT1/3/6, PARP1	Senomorphic & Metabolic Support	**Level I**	500–1,000 mg NMN or 300–600 mg NR daily	Replenishes cellular NAD+; restores mitochondrial quality control; reduces cellular DNA damage and oxidative stress.	Nature 2026
Vitamin D3 + K2	Telomere maintenance, NF-κB	Senomorphic & Genomic Protector	**Level I** (Human RCT)	2,000–5,000 IU D3 + 100–200 mcg K2 daily	2,000 IU/day slowed leukocyte telomere shortening significantly; modulates systemic inflammatory tone.	Harvard Gazette 2025
Magnesium Chelate	PARP1 repair, NMDA,	Micronutrient / Genome Stability	**Level I**	300–400 mg elemental	Essential for double-strand DNA repair machinery;	Nature 2026

Compound / Modality	Target SCAP / Pathway	Classification	Evidence Level	Clinical / Standard Dose	Primary Human & Preclinical Findings	Key Scientific Citations
	Telomere stability			magnesium daily	improves sleep architecture and nocturnal cellular regeneration.	
Rapamycin (Sirolimus)	mTORC1 selective allosteric inhibition	Senomorphic (Prescription)	**Level I / II**	5–10 mg once weekly (Pulse)	Suppresses IL-1α translation and complete SASP secretome; restores stem cell function; may interfere with exercise if unpulsed.	PMC 13082878
Resistance Training	Muscle p16INK4a, p21CIP1, AMPK	Senolytic / Senomorphic Lifestyle	**Level I**	3–4 sessions/week (45–60 min)	Directly lowers p16INK4a and SASP markers in human skeletal muscle biopsies; stimulates satellite cell rejuvenation.	PMC 12181168, PMC 9970302
Time-Restricted Feeding (16:8)	AMPK, TFEB, Insulin / IGF-1 axis	Autophagic / Senomorphic Lifestyle	**Level I**	16–18 hour daily fasting window	Suppresses systemic TNF-α, IL-6, and lipid peroxides; elevates autophagic flux; preserves lean body mass.	PMC 10940141, MDPI Nutrients 2026

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6. BIOMARKERS & CLINICAL MONITORING FRAMEWORK

Tracking senescent cell burden and protocol efficacy requires a combination of circulating inflammatory panels, metabolic indicators, and advanced cellular longevity assays.

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|                          SENESCENCE CLINICAL BIOMARKER PANELS                                   |
|                                                                                                 |
|  [ PRIMARY CIRCULATING SASP ]   --->   IL-6, GDF15, TNF-α, hs-CRP, PAI-1, MCP-1 (CCL2)          |
|  [ METABOLIC & ORGANS ]         --->   Fasting Insulin, HOMA-IR, HbA1c, ALT/AST, Cystatin-C      |
|  [ MOLECULAR / EPIGENETIC ]     --->   p16INK4a Expression (PBMC/Skin), DNAm PhenoAge / GrimAge  |
+-------------------------------------------------------------------------------------------------+
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6.1 Circulating Inflammatory & SASP Biomarkers

- **Growth Differentiation Factor 15 (GDF15):** Identified as a robust circulating proteomic biomarker of human cellular senescence and mitochondrial stress [PMC 12181601, Mayo Clinic]. Serum elevations correlate with whole-body senescent cell burden, chronic disease progression, and all-cause mortality.
- **Interleukin-6 (IL-6):** The primary pro-inflammatory cytokine of the SASP secretome [PMC 12181601, Mayo Clinic]. Serves as a dynamic indicator of systemic NF-κB and SASP silencing.
- **High-Sensitivity C-Reactive Protein (hs-CRP):** Systemic hepatic biomarker reflecting downstream IL-6 and IL-1β stimulation. Optimal target: **<0.5 mg/L**.
- **Plasminogen Activator Inhibitor-1 (PAI-1 / Serpine-1):** Key pro-thrombotic and pro-fibrotic SASP marker regulated downstream of p53/p21.
- **Tumor Necrosis Factor-Alpha (TNF-α) & Monocyte Chemoattractant Protein-1 (MCP-1 / CCL2):** Drivers of chronic macrophage infiltration and tissue inflammaging.

6.2 Epigenetic, Telomeric, and Transcriptomic Clocks

- **DNA Methylation Clocks (Horvath DNAmAge, PhenoAge, GrimAge v2, DunedinPACE):** Quantify biological pace of aging and biological age acceleration based on CpG methylation patterns influenced by senescence accumulation.
- **Leukocyte Telomere Length (LTL) Assays:** Quantifies average telomeric repeat length; longitudinal preservation reflects telomeric protection [Harvard Gazette 2025].

6.3 Direct Tissue & PBMC Markers: p16INK4a and p21CIP1

- **Peripheral Blood Mononuclear Cell (PBMC) p16INK4a mRNA Expression:** Quantitative RT-PCR testing of T-lymphocyte p16INK4a expression provides a direct metric of cellular senescence burden in circulating immune compartments.
- **Skin Punch Biopsy / Adipose Tissue Biopsy:** Utilized in clinical research trials to stain for senescence-associated beta-galactosidase (SA-β-gal) activity and p16INK4a/p21CIP1 immunohistochemistry [PMC 6796530].

6.4 Baseline & Tracking Schedule

To accurately assess protocol response, adhere to the following testing timeline:

Timepoint	Panel	Purpose
Baseline (Day 0)	Comprehensive metabolic panel, GDF15, IL-6, hs-CRP, fasting insulin/lipids, optional epigenetic clock	Establish individual baseline senescence and inflammatory burden
30 Days	hs-CRP, ALT/AST, renal function, metabolic markers	Early safety and tolerance check; detect any hepatic or renal signal
90–180 Days (post 3–6 pulse cycles)	Full SASP panel (GDF15, IL-6, TNF-α, PAI-1), PBMC p16INK4a (if available), repeat biological age clock	Assess efficacy; compare biological age trajectory

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7. IMPORTANT LIMITATIONS, CONTRAINDICATIONS, AND CLINICAL CAVEATS

While senolytic and senomorphic therapies represent a promising paradigm in longevity medicine, premature or unguided implementation carries significant biological risks. This section is essential reading before any protocol is initiated.

7.1 Beneficial Physiological Roles of Senescent Cells

Senescence is not exclusively pathological; it evolved as an essential biological adaptation:

1. **Wound Healing & Tissue Regeneration:** Acute senescent fibroblasts and endothelial cells secrete PDGF-AA and localized SASP factors that stimulate myofibroblast differentiation and orchestrate tissue repair. Complete elimination of acute senescent cells delays cutaneous wound closure.
2. **Tumor Suppression:** Acute oncogene-induced senescence (OIS) prevents premalignant cells harboring oncogenic mutations (*RAS*, *BRAF*) from replicating into aggressive neoplasms.
3. **Placental Development & Embryogenesis:** Senescent signaling plays a defined role in embryonic patterning and maternal-fetal vascular remodeling.
4. **Clinical Caveat:** *Senolytic pulse therapy should be strictly avoided during active acute wound healing, following major surgical procedures, or during pregnancy and lactation.*

7.2 Drug-Drug Interactions & Hepatic Clearance (CYP3A4/CYP2C9)

High-dose bioflavonoids and pharmaceutical senolytics interact with hepatic cytochrome P450 isoenzymes:

- **CYP3A4 & CYP2C9 Inhibition:** High-dose Quercetin and Fisetin inhibit CYP3A4 and CYP2C9, altering the clearance and elevating serum concentrations of medications including anticoagulants (Warfarin, DOACs), statins (Atorvastatin, Simvastatin), calcium channel blockers, and immunosuppressants.
- **Antiplatelet Activity:** High-dose bioflavonoids possess mild antiplatelet and antithrombotic properties. Concurrent use with Aspirin, Clopidogrel, or NSAIDs increases bleeding risks.
- **Practical Guidance:** Review all prescription medications with a physician or pharmacist before initiating any pulse protocol. Where interaction risk exists, consider dose reduction of the interacting drug or substitution of the senolytic agent.

7.3 Intermittent vs. Chronic Toxicity Concerns

- **Avoid Continuous High-Dose Senolytics:** Taking high-dose Fisetin, Quercetin, or synthetic BCL-2 inhibitors daily without interruption can lead to off-target cytotoxicity, apoptosis in healthy stem cell populations, persistent thrombocytopenia, and gastrointestinal mucosal injury. **Strict adherence to the 2-day monthly pulse model is mandatory.**
- **Monitoring During Pulse Days:** Watch for unusual fatigue, bruising, bleeding gums, or gastrointestinal upset. Discontinue and consult a physician if these occur.

7.4 Exercise-Compound Interference (The mTOR vs. AMPK Paradox)

- **The Antioxidant / mTOR Blunting Window:** High doses of concentrated polyphenols (Curcumin, Resveratrol, Quercetin) or mTOR inhibitors (Rapamycin) taken immediately pre- or post-workout neutralize exercise-induced reactive oxygen species (ROS). These transient ROS bursts are essential signaling triggers for muscular adaptation, mitochondrial biogenesis, and hypertrophy.
- **Timing Rule:** Administer base polyphenol supplements and rapamycin at least **2 to 3 hours apart from resistance training sessions** to ensure unhindered muscle protein synthesis and adaptive remodeling [PMC 13082878].

7.5 Evidence Limitations — Read Before Beginning

- **Much of the human senolytic evidence is preliminary.** Several key human trials are open-label, single-arm, or pilot studies with small sample sizes. Placebo-controlled, long-term outcome data remain limited.
- **Species translation is imperfect.** Many mechanistic claims derive from murine models; dosing, pharmacokinetics, and tissue distribution differ substantially in humans.
- **Senolytic dosing is not standardized.** The regimens presented here reflect published trial protocols and widely cited translational recommendations, not regulatory approvals.
- **This document is educational and is not medical advice.** Anyone considering prescription compounds (Dasatinib, Rapamycin) or high-dose supplement pulses should do so under qualified clinical supervision with appropriate baseline and follow-up laboratory monitoring.

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8. THE PRINTABLE DAILY & INTERMITTENT CHECKLIST

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                THE ZOMBIE CELL ELIMINATION CHECKLIST: DAILY & MONTHLY LOG
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[ ] MORNING ROUTINE (06:00 - 11:30)
    [ ] 15-30 min Direct Sunlight (No sunglasses) within 1 hour of waking
    [ ] 500-750 mL Water + 1/4 tsp Sea Salt + Lemon Juice
    [ ] Optional: 1-2 Cups Organic Green Tea (Matcha/Sencha) or Black Coffee (Autophagy Priming)
    [ ] Maintain Fast (Water, electrolytes, unsweetened tea/coffee only)
    [ ] Morning Base Stack:
        [ ] NMN (500-1,000 mg) OR NR (300-600 mg)
        [ ] Spermidine (1.2-3.0 mg) [PMC 5807086]
        [ ] Trans-Resveratrol (250-500 mg)
        [ ] Bioavailable Curcumin (500-1,000 mg)

[ ] AFTERNOON ROUTINE (11:30 - 17:30)
    [ ] Break-Fast Meal (11:30-12:30) - Time-Restricted Feeding (16:8 Protocol) [PMC 10940141]
    [ ] Senolytic Food Checklist (Incorporate at least 3-4 items):
        [ ] Fresh Strawberries / Organic Apples (Fisetin source)
        [ ] Red Onions / Capers / Kale (Quercetin source)
        [ ] Wild Blueberries / Blackberries (Anthocyanins)
        [ ] Broccoli Sprouts (Sulforaphane / Nrf2 activation)
        [ ] Extra Virgin Olive Oil (Cold-Pressed, 1-2 tbsp)
        [ ] Shiitake Mushrooms / Natto / Aged Cheese (Spermidine)
    [ ] Exercise Session (14:00-16:30):
        [ ] Resistance Training (Compound Lifts, 45-60 min) [PMC 12181168, PMC 9970302]
        [ ] OR HIIT Conditioning (4-6 Sprint Intervals) / Zone 2 Aerobic Base
    [ ] Post-Workout Recovery Meal (High protein, polyphenol rich)
    [ ] Close Feeding Window by 17:30-18:30 (Initiate 16-18h overnight fast)

[ ] EVENING & PRE-SLEEP ROUTINE (17:30 - 22:30)
    [ ] Conclude dinner at least 3-4 hours prior to sleep
    [ ] Eliminate overhead blue/bright LED lighting; wear blue-blocking glasses
    [ ] 10-15 min Stress Down-Regulation (Box Breathing, Parasympathetic Reset, Meditation)
    [ ] Evening Micronutrient & Sleep Stack (60 min pre-sleep):
        [ ] Vitamin D3 (2,000-5,000 IU) + Vitamin K2 (100-200 mcg MK-7) [Harvard Gazette 2025]
        [ ] High-Absorption Magnesium Glycinate/L-Threonate (300-400 mg)
        [ ] L-Theanine (200 mg) + Apigenin (50 mg)
    [ ] Optimize Sleep Environment: Bedroom temp 65°F-68°F (18°C-20°C), pitch black
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    [ ] Target: 7.5 - 9.0 Hours Restorative Sleep (Glymphatic clearance active)

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                      MONTHLY & INTERMITTENT PULSE TRACKING SHEET
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[ ] MONTHLY HIGH-DOSE FISETIN PULSE (Cycle Day 1 & Day 2 of Month)
    [ ] Day 1: Fisetin ~20 mg/kg with healthy fat meal (e.g., 1,400-1,800 mg) [PMC 6197652]
    [ ] Day 2: Fisetin ~20 mg/kg with healthy fat meal (e.g., 1,400-1,800 mg) [PMC 6197652]
    [ ] Days 3-30: Resume Daily Base Protocol

[ ] OPTIONAL QUERCETIN PULSE (Alternative / Complementary)
    [ ] Quercetin 1,000-1,250 mg/day for 2-3 consecutive days [PMC 6796530]

[ ] PRESCRIPTION D+Q PROTOCOL (Physician-Supervised Only)
    [ ] Dasatinib 100 mg + Quercetin 1,000-1,250 mg/day for 2-3 days [PMC 6796530]
    [ ] Cycle repeated every 14-28 days as clinically directed

[ ] WEEKLY 24-HOUR FAST (Once Per Week, e.g., Sunday 18:00 to Monday 18:00)
    [ ] 24-Hour Water + Electrolyte Deep Autophagic Window Completed

[ ] WEEKLY RAPAMYCIN PULSE (If Prescribed) — Schedule on Complete Rest Day
    [ ] 5-10 mg once weekly, NOT within 24-48 hours of resistance training [PMC 13082878]

[ ] PERIODIC BIOMARKER LOG (Every 90-180 Days)
    [ ] Baseline Date: _____________  |  GDF15: _________ pg/mL  |  IL-6: _________ pg/mL
    [ ] Follow-Up Date: _____________  |  hs-CRP: ________ mg/L   |  HbA1c: ________ %
    [ ] Biological Age / Notes: ____________________________________________________
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Quick-Reference Card (Cut Out & Post)

Time	Action	Core Rationale
Wake	Sunlight 15–30 min	Circadian alignment; nocturnal autophagy
Morning	Fast + electrolytes + base stack	AMPK activation; SASP silencing
11:30–12:30	First meal + polyphenols	Senolytic food matrix; nutrient sensing
14:00–16:30	Resistance training / HIIT	p16INK4a reduction; mitochondrial biogenesis
17:30–18:30	Final meal (close window)	Insulin decline; autophagic transition
Evening	Stress down-regulation + sleep stack	Vagal tone; DNA repair cofactors
Night	7.5–9 h sleep, cool & dark	Glymphatic clearance; hormonal repair
Monthly	Fisetin 20 mg/kg × 2 days	Intermittent senolytic clearance

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