EP53-R9 as a Safety Platform for Epigenetic Rejuvenation

An Orthogonal Tumor Suppression Strategy
for Partial Reprogramming

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Marko Leicht
Independent Researcher — Obertrubach, Germany
marko.leicht@arcor.de

Revision note: This v2 incorporates the evolving landscape of safe reprogramming strategies (chemical reprogramming, OSK-only approaches, ER-100 clinical trial) and integrates key supporting evidence from Menendez et al. (2012, *Aging Cell*), demonstrating that increased p53 dosage preserves pluripotency while reducing tumorigenicity.

1. The Unresolved Safety Gap

Partial epigenetic reprogramming via Yamanaka factors (OSKM/OSK) has demonstrated the ability to reverse biological age markers in vitro and in vivo. However, this process relies fundamentally on the suppression of the p53 tumor suppressor pathway. This creates a direct mechanistic tension: the same pathway that enables rejuvenation simultaneously increases cancer risk.

In January 2026, Life Biosciences received FDA clearance for ER-100 (NCT07290244), the first human clinical trial of epigenetic reprogramming — a landmark moment. ER-100 uses OSK (without c-Myc) delivered via AAV to the eye for optic neuropathies. Yet even this pioneering trial sidesteps rather than solves the p53 safety problem: it uses a local injection into an immune-privileged organ with limited proliferative capacity.

For the field's ultimate goal — systemic rejuvenation — the safety question remains unanswered. In vivo OSKM expression causes intestinal and hepatic failure as the primary cause of lethality (Pico et al., 2025), and no current approach actively strengthens tumor defense during or after reprogramming.

2. The Evolving Landscape: Alternative Safety Strategies

The field has developed several strategies to mitigate reprogramming-associated risks. Understanding these is essential for positioning EP53-R9 within the broader context:

Cyclic Induction Protocols

Pulsed OSKM expression (e.g., 2 days ON / 5 days OFF) allows safe long-term reprogramming for up to 35 cycles. This reduces but does not eliminate cancer risk, as it relies on timing rather than active tumor suppression.

OSK Without c-Myc

Removing the known oncogene c-Myc from the Yamanaka cocktail reduces tumorigenicity. ER-100 and the Sinclair lab's AAV9-OSK approach use this strategy. However, p53 suppression still occurs with OSK alone, and reprogramming efficiency is reduced.

Chemical Reprogramming (7c Cocktail)

The 7c small-molecule cocktail (Repsox, trans-2-phenylcyclopropylamine, DZNep, TTNPB, CHIR99021, Forskolin, Valproic acid) achieves epigenetic rejuvenation through a fundamentally different pathway than OSKM. Critically, multi-omics analysis shows that 7c **upregulates** the p53 pathway (Mitchell et al., 2024, *eLife*), in stark contrast to OSKM's suppression. A 2025 study in *EMBO Molecular Medicine* demonstrated lifespan extension in C. elegans. Chemical reprogramming is promising but remains early-stage and may not achieve the depth of rejuvenation possible with transcription factor-based approaches.

Senescence-Targeted Reprogramming

Driving OSK expression from the Cdkn2a promoter restricts reprogramming to aged and stressed cells only, reducing off-target effects. This elegant approach limits scope but does not actively protect against oncogenic transformation within targeted cells.

SB000 (Shift Bioscience)

A single, undisclosed gene target claimed to rejuvenate cells without activating pluripotency pathways, with twice the methylome rejuvenation of OSKM (bioRxiv, June 2025). The gene identity remains proprietary and the work is not yet peer-reviewed, limiting independent evaluation.

> *Each of these strategies addresses the safety problem from a different angle. None, however, provides an active, orthogonal tumor suppression mechanism that operates independently of the nuclear reprogramming process itself. This is the specific niche that EP53-R9 occupies.*

3. The Insight: EP53-R9 as an Orthogonal Safety Layer

Elephant TP53-Retrogene 9 (EP53-R9) encodes a C-terminally truncated p53 protein that induces apoptosis through a transcription-independent mitochondrial pathway (Abegglen et al., 2023, *Cell Death Discovery*). Key mechanistic features:

- **Mitochondrial localization:** EP53-R9 binds the chaperone protein Tid1, which mediates its translocation to the mitochondrial outer membrane.
- **No nuclear activity:** EP53-R9 lacks the nuclear localization signal (NLS) and oligomerization domain of full-length p53. It does not function as a nuclear transcription factor.
- **Direct apoptosis induction:** At the mitochondria, EP53-R9 binds pro-apoptotic Bax, triggering cytochrome c release and caspase activation.
- **Functional in human cells:** Demonstrated apoptotic activity in U2OS human osteosarcoma cells. EP53-loaded lipidoid nanoparticles are in preclinical development for NSCLC and colon cancer (AACR 2025).

The Core Argument

Because EP53-R9 operates exclusively at the mitochondria and does not enter the nucleus, it should not interfere with the nuclear epigenetic remodeling driven by OSK/OSKM. This creates the possibility of simultaneous rejuvenation (nuclear) and tumor surveillance (mitochondrial) — two orthogonal processes in two separate cellular compartments.

4. Key Supporting Evidence: The Menendez et al. Precedent

The strongest experimental support for this concept comes from a study by Serrano, Belmonte and colleagues:

> *Menendez S, Camus S, et al. (2012). "Increased dosage of tumor suppressors limits the tumorigenicity of iPS cells without affecting their pluripotency." Aging Cell, 11(1), 41–50.*

This study demonstrated three critical findings:

- **Pluripotency preserved:** iPSCs with an extra copy of p53 or Ink4a/ARF showed normal pluripotency in both in vitro and in vivo differentiation assays.

- **Tumorigenicity reduced:** The same cells showed improved engagement of the p53 pathway during teratocarcinoma formation, leading to reduced tumorigenic potential.
- **Drug responsiveness enhanced:** Extra-p53 iPSCs showed improved response to anticancer drugs, providing a secondary elimination mechanism.

> *The logical extension is straightforward: If full-length nuclear p53 in extra copies does not block reprogramming, then EP53-R9 — which lacks nuclear localization entirely and operates only at the mitochondria — should be at least equally compatible with reprogramming, while providing an additional, mechanistically distinct layer of tumor suppression.*

Notably, this combination has never been tested. Prof. Manuel Serrano (Altos Labs, co-author of the 2012 study and creator of the super-p53 mouse model) confirmed in personal communication (February 2026) that reprogramming in a super-p53 background has not been performed: “We never tested reprogramming in mice with extra p53. These are expensive and lengthy experiments.”

5. Proposed Experimental Design

Phase 1: In Vitro Proof-of-Concept (3–4 months)

The critical first experiment is designed to be fast, inexpensive, and decisive:

- **Cell system:** Human dermal fibroblasts (aged donor, passage >10)
- **EP53-R9 delivery:** Transient transfection with EP53-R9 mRNA (avoids genomic integration, mimics therapeutic scenario)
- **Reprogramming:** Transient OSK expression (non-integrating, 4–7 days)
- **Readouts:** (a) Epigenetic age (Horvath clock / PC-based clocks), (b) p53 pathway markers (p21, MDM2), (c) Apoptosis rate in EP53-R9+ vs. control cells upon DNA damage challenge, (d) Colony formation assay (teratoma proxy), (e) Confirmation of EP53-R9 mitochondrial localization (IF/confocal)

Key comparison groups: (1) OSK alone, (2) OSK + EP53-R9, (3) EP53-R9 alone, (4) Untreated control. The decisive question: Does group 2 show equivalent epigenetic age reversal to group 1, with reduced colony formation and enhanced apoptotic response to DNA damage?

Phase 2: In Vivo Validation (12–18 months, contingent on Phase 1)

If Phase 1 confirms compatibility, the in vivo experiment becomes justified:

- Transgenic mice with inducible EP53-R9 (Tet-ON, safe harbor locus — the Schiffman lab has already generated this model)
- Cross with OSKM/OSK-inducible mice (available at multiple institutions)
- Cyclic reprogramming protocol (2d ON / 5d OFF) with and without EP53-R9 induction
- Endpoints: Epigenetic age, tumor incidence, organ-specific toxicity (especially intestinal and hepatic), lifespan

6. Strategic Positioning

EP53-R9 does not compete with existing safety strategies — it complements them. The following table positions EP53-R9 within the current landscape:

Approach	Mechanism	p53 Status	Active Tumor Defense?	Limitation
OSKM (standard)	4-factor nuclear reprogram.	Suppressed	No	Cancer risk, organ toxicity
OSK (no c-Myc)	3-factor nuclear reprogram.	Suppressed	No	Lower efficiency, p53 still down
Cyclic induction	Pulsed OSKM/OSK	Intermittent	No (passive)	Timing-dependent, no kill-switch
7c chemical	Small molecule cocktail	Upregulated	Partial (endogenous)	Early-stage, weaker rejuvenation?
Cdkn2a-targeted OSK	Senescent-cell-specific	Suppressed locally	No	Limited scope
SB000	Single gene (undisclosed)	Unknown	Unknown	Proprietary, not peer-reviewed
EP53-R9 + OSK/OSKM	**Mitochondrial apoptosis + nuclear reprogram.**	**Orthogonal**	**Yes (mitochondrial)**	**Untested (this proposal)**

7. What This Experiment Would Prove

The proposed in vitro experiment has a binary outcome, and both results are publishable:

If EP53-R9 is compatible with OSK-driven rejuvenation: This establishes the first orthogonal safety layer for epigenetic reprogramming — a mitochondrial tumor suppression mechanism that operates independently of nuclear reprogramming. This would have immediate implications for the design of systemic rejuvenation therapies, where local delivery (as in ER-100) is not an option.

If EP53-R9 blocks or reduces rejuvenation: This reveals an unexpected mitochondrial involvement in epigenetic reprogramming, challenging the assumption that OSK-driven rejuvenation is a purely nuclear process. Given the growing evidence for mitochondrial-nuclear crosstalk in aging (mitochondrial retrograde signaling, NAD+ metabolism), this would be a mechanistically significant finding in its own right.

> *Either outcome advances the field. The experiment is fast (3–4 months), inexpensive relative to in vivo models, and addresses a question that — as confirmed by Prof. Serrano — no laboratory has yet investigated.*

8. Combinability with Existing Approaches

A key advantage of EP53-R9 is that it does not require replacing existing strategies. It can be layered on top of any reprogramming approach as additional insurance:

- **EP53-R9 + cyclic OSKM:** Active tumor defense during the ON-phases, when p53 is suppressed and cells are most vulnerable to transformation.
- **EP53-R9 + full OSKM (including c-Myc):** Could enable more aggressive reprogramming protocols that are currently too risky, by providing a mitochondrial kill-switch for aberrant cells.
- **EP53-R9 + 7c chemical reprogramming:** Dual safety layer — endogenous p53 upregulation (nuclear) plus exogenous EP53-R9 (mitochondrial). A “belt and suspenders” approach attractive for regulatory approval.
- **EP53-R9 for gut and liver specifically:** Tissue-targeted EP53-R9 expression in intestinal and hepatic cells could address the primary cause of in vivo reprogramming lethality (Pico et al., 2025).

9. Ideal Collaborators

Researcher	Institution	Relevance
Lisa Abegglen / Joshua Schiffman	Huntsman Cancer Institute, Univ. of Utah	EP53-R9 discoverers; have transgenic mouse models with inducible EP53-R9; developing EP53 nanoparticle therapeutics
Manuel Serrano	Altos Labs, UK	Super-p53 mice; 2012 proof that extra p53 preserves pluripotency; confirmed the blind spot (personal comm. 2026)
Juan Carlos Izpisúa Belmonte	Altos Labs, US (formerly Salk Institute)	Co-author of Menendez 2012; pioneer of in vivo partial reprogramming
David Sinclair	Harvard Medical School / Life Biosciences	OSK-based rejuvenation in mice; ER-100 clinical trial; would benefit from safety layer for systemic extension
Vittorio Bhatt / George Church	Harvard / Colossal Biosciences	2024 CRISPR-KO of all 29 TP53 retrogenes in elephant cells; deep expertise in elephant p53 biology

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