Artificial intelligence–assisted reinterpretation of preclinical progeria research suggests a hierarchical nuclear–vascular resilience framework with translational implications

Paolo Madeddu, MD;[1] Monica Cattaneo, PhD;[2] Annibale Alessandro Puca, MD.[2,3]

1. Experimental Cardiovascular Medicine, Bristol Heart Institute, University of Bristol, Bristol, U.K.
2. Cardiovascular Department, IRCCS MultiMedica, Milan, Italy.
3. Department of Medicine, University of Salerno, Italy

Corresponding author:
Paolo Madeddu
Bristol Heart Institute,
University of Bristol,
Bristol, U.K.
Email: mdprm@bristol.ac.uk

Abstract

Preclinical research traditionally advances through hypothesis-driven experimentation that establishes mechanistic pathways to support translational development. While this approach has generated major biological insights, it may underemphasize alternative organizational patterns embedded within complex datasets, particularly in rare diseases where opportunities for experimental reiteration are limited. Recent advances in conversational artificial intelligence (AI) provide an opportunity to support structured analytical dialogue as a complementary approach for re-examining validated experimental observations.

Here, we evaluated the feasibility and informative value of an investigator-led structured analytical dialogue to reinterpret a previously published preclinical study of Hutchinson–Gilford Progeria Syndrome (HGPS), a rare disorder characterized by accelerated cardiovascular aging. Investigators defined the analytical questions, established interpretative boundaries, and critically evaluated successive AI-generated outputs, while the AI platform functioned exclusively as an analytical support tool for exploring complementary conceptual organization of experimentally validated findings. The original study showed that delivery of the longevity-associated *LAV-BPIFB4* gene preserved left ventricular diastolic function, reduced perivascular fibrosis, increased coronary arteriole density, and attenuated cellular senescence without modifying progerin accumulation. Structured analytical dialogue generated complementary hierarchical interpretations of these observations. By integrating graphical dispersion with individual-level numerical data, the investigator-led dialogue identified heterogeneous response trajectories and suggested that cardiovascular protection may be viewed as emerging from coordinated interactions between nuclear stress adaptation and vascular remodelling within a broader resilience framework. These interpretations are presented as hypothesis-generating conceptual extensions rather than new experimental findings.

This study demonstrates the feasibility of structured investigator-led analytical dialogue as a complementary methodological approach for broadening interpretation of existing preclinical datasets while preserving the original experimental evidence. By making analytical reasoning more transparent and explicitly distinguishing validated observations from conceptual reinterpretation, this framework may assist prioritization of future mechanistic investigations, particularly in rare cardiovascular diseases where maximizing insight from existing datasets is especially important.

Keywords

Hutchinson–Gilford Progeria Syndrome; Cardiovascular aging; Artificial intelligence–assisted reinterpretation; Human–AI analytical dialogue; Translational medicine

Introduction

Biological research has traditionally progressed through a cognitive process in which theory informs hypothesis generation, experiments test cause-and-effect relationships, and mechanistic hierarchies are validated through targeted genetic or molecular perturbations, including transcriptional and post-transcriptional modulation. This framework has enabled major advances in biomedical science by providing a rigorous basis for establishing causal mechanisms and translating experimental discoveries into therapeutic strategies. Nevertheless, because hypothesis-driven investigation is inherently guided by predefined conceptual models, alternative biological relationships may remain underexplored.[1] Re-examining validated experimental observations from alternative conceptual perspectives may generate complementary biological hypotheses and identify organizational patterns that were not emphasized in the original analysis, thereby helping to prioritize subsequent experimental investigation.

Artificial intelligence (AI) has increasingly contributed to translational cardiovascular medicine through applications such as imaging interpretation, predictive modelling, risk stratification, and automated signal assessment.[2-5] The recent availability of conversational large language models (LLMs), capable of generating responses to free-text queries without task-specific programming, has created new opportunities for interactive analytical dialogue between investigators and AI.[6] Their broad applicability has stimulated growing interest in clinical practice, medical education, and scientific research, while also raising important questions regarding reliability and the need for appropriate human oversight.[7-9] The use of LLM platforms may be particularly valuable for advancing translational and clinical research on rare genetic diseases, where limited cohort sizes amplify the importance of extracting maximal information from existing datasets.[10]

Hutchinson–Gilford Progeria syndrome (HGPS), caused by a mutation in the *LMNA* gene leading to progerin accumulation, is characterized by accelerated vascular aging, progressive arterial stiffening, and premature cardiovascular mortality.[11,12] Current therapies that reduce progerin production or accumulation modestly extend survival but do not prevent the progressive cardiovascular complications that ultimately drive early death.[13] These limitations highlight the need for adjunct strategies targeting downstream vascular dysfunction and maladaptive cardiac remodelling.

Several gene variants enriched in long-lived human populations may represent biological counterparts to progeroid mutations because they are associated with enhanced stress resilience,

vascular homeostasis, and resistance to cellular senescence. Within this framework, we proposed that horizontal transfer of a healthy longevity-associated gene might mitigate both physiological and progeroid cardiovascular senescence. A longevity-associated variant (LAV) of the *BPI fold containing family B member 4 (BPIFB4)* gene has been linked to exceptional human healthspan and cardiovascular protection,[14] and its viral vector-mediated delivery attenuated cardiovascular aging in aged mice.[15] Building on this rationale, we recently investigated the same strategy in a murine model of HGPS, where *LAV-BPIFB4* delivery preserved diastolic left ventricular function, reduced perivascular fibrosis, and attenuated senescence signalling without altering progerin accumulation.[16] These findings supported an intervention-anchored interpretation in which *LAV-BPIFB4* delivery was associated with adaptive changes in cardiac microvasculature and ventricular relaxation.

The present study examined the feasibility and informative value of a structured analytical dialogue in which investigators used AI to extend the interpretation of findings from the previously published HGPS study.[16] Through qualitative assessment of graphical data distributions and cross-examination of individual-level numerical data, we explored whether the observations could be reorganized to generate additional mechanistic and translational hypotheses.

Methods

AI reporting and conceptual framework

The analytical dialogue was conducted using ChatGPT (GPT-5.5; OpenAI, San Francisco, CA, USA) accessed through the ChatGPT web interface under continuous investigator supervision. The analytical workflow was applied after publication of the original study and therefore functioned as a post-publication analytical reinterpretation rather than as part of the original experimental design. The analytical sessions were performed between February and June, 2026. The model was used in its standard conversational configuration without domain-specific fine-tuning, external biomedical knowledge-base integration, or retrieval-augmented generation. Because the model is proprietary and may evolve, reproducibility was supported by documenting representative prompts, analytical workflow, decision criteria, and reconstructed dialogue in **Supplementary Data 1**, rather than by relying solely on model version identification.

Investigator-AI dialogue, boundaries, and responsibilities

The open-access PDF version of the published article from the journal website was uploaded on the ChatGPT platform. Individual-level numerical data, including echocardiography and histology endpoints from the *in vivo* study on HGPS mice and controls, and results from cell biology experiments, were supplied as dispersion images and tables. No unpublished data, annotations or external datasets were provided.

Investigators retained full responsibility for defining the analytical questions, directing the dialogue, critically evaluating AI-derived interpretations, and determining the concepts to be incorporated into the final interpretative framework.

Core analytical questions included:

• Can validated phenotypic outcomes be reorganized into alternative hierarchical frameworks without contradicting the original conclusions?

• Do dispersion patterns and extreme datapoints suggest context-dependent responsiveness beyond group-level averages?

• Does the absence of linear scaling between cardiac BPIFB4 expression and outcome support a threshold model of pathway engagement?

• What are the implications of cardiac BPIFB4 levels reflecting both endogenous and transgenic protein?

• Can vascular remodelling and senescence attenuation by *LAV-BPIFB4* delivery be interpreted as upstream regulatory layers rather than solely downstream effects?
• What translational implications emerge if vascular architecture and nuclear stress adaptation are viewed as resilience nodes?

Analytical boundaries were explicitly defined before starting the study. AI functioned exclusively as an analytical support tool to explore alternative hierarchical organization of biological processes already supported by the published data, identify complementary conceptual entry points, and propose biologically plausible mechanistic interpretations. AI-generated interpretations were retained only when they were fully consistent with the published dataset, biologically plausible, and supported by the existing scientific literature. Generation of new experimental claims, probabilistic predictions, statistical analyses, or causal assertions extending beyond the original evidence was excluded. When alternative or conflicting interpretations emerged, investigators reformulated the analytical question and repeated the dialogue until the proposed interpretations could be reconciled with the predefined analytical boundaries or were discarded. Accepted interpretations were further examined through a continuous validation loop grounded in established knowledge of HGPS pathophysiology and relevant published literature.

Bias mitigation and reduction of directional influence

Because investigator-defined prompts inherently introduce the possibility of interpretative bias, several procedural safeguards were incorporated into the analytical workflow. First, prompts were structured around predefined analytical questions rather than desired outcomes, encouraging evaluation of alternative hierarchical interpretations independent of the original intervention-anchored framework. Second, orthogonal conceptual exploration required reinterpretation of identical observations from distinct biological entry points, thereby introducing a deliberate counter-perspective designed to reduce anchoring effects. Third, a dialogue-verification step ensured that prompts were accurately reconstructed and interpreted, minimizing drift toward implicit investigator assumptions. Finally, integration of dispersion-focused analysis — including examination of extreme datapoints and individual-level variability — shifted interpretation away from mean-based narratives that can reinforce prior expectations. Collectively, these measures were intended to reduce directional influence and to maintain analytical transparency within an investigator-led framework.

Diagrammatic representation and graphical implementation

Diagrams were manually developed using BioRender (Toronto, Canada). The structured analytical dialogue informed their conceptual organization, whereas graphical implementation remained entirely under investigator control. Completed figures were subsequently reviewed during the analytical dialogue with the AI interface to confirm consistency with the evolving interpretative framework.

Criteria ensuring methodological transparency and reproducibility

Supplementary Data 1 provides the analytical workflow, glossary of methodological terminology, representative investigator prompts, decision criteria, and a reconstructed analytical transcript documenting the evolution of the investigator–AI dialogue. In addition, **Figure 1** summarizes the investigator-led analytical workflow.

Results

Previously published experimental observations

The previously published study subjected to structured analytical reinterpretation investigated whether *LAV-BPIFB4* gene transfer could attenuate early manifestations of cardiomyopathy in a transgenic mouse model of HGPS.[16]

As previously reported, therapeutic gene delivery increased BPIFB4 protein levels in the heart. Because the antibody used for protein detection does not distinguish murine from human BPIFB4, the measured increase reflected total cardiac BPIFB4 protein. This molecular change was associated with preserved left ventricular diastolic function, reduced perivascular fibrosis, increased coronary arteriole density, and reduced markers of cellular senescence.

Parallel experiments performed in fibroblasts derived from patients with HGPS showed that LAV-BPIFB4 transduction attenuated senescence and profibrotic signalling without modifying progerin levels. Transgene delivery reduced the expression of p53 and p21, crucial proteins in the stress response activated by progerin accumulation,[17] and decreased the ability of HGPS fibroblasts to secrete epiregulin (EREG), a long-acting ligand of epidermal growth factor receptor (EGFR) implicated in profibrotic remodelling.[18] In line with the *in vitro* data, *LAV-BPIFB4* delivery reduced EREG levels in the heart of HGPS mice.

These experimental observations supported an intervention-anchored framework in which *LAV-BPIFB4* delivery constituted the initiating event in a linear cause–effect cascade downstream to progerin. Improvements in microvascular architecture and extracellular matrix composition were interpreted as intermediate structural mediators linking molecular pathway activation to improved left ventricular relaxation. However, the original interpretation did not explicitly examine alternative hierarchical organization among the validated observations.

AI-assisted reasoning provides complementary conceptual trajectories

AI-assisted analysis suggested that identical phenotypic outcomes could be organized into complementary hierarchical trajectories depending on the analytical entry point.

As shown in **Figure 2,** the complementary hierarchical interpretation suggested that the increased arteriole density and reduced perivascular fibrosis are candidate upstream determinants of functional improvement. Moreover, the reduction in p21 and other senescence markers advised

that, by restoring nuclear stress adaptation, LAV-BPIFB4 could dampen pro-fibrotic signalling and create conditions favorable for vascular remodelling.

The AI-assisted dialogue also suggested that the above-mentioned vascular-first and nuclear stress–senescence perspectives are complementary hierarchical emphases rather than competing mechanisms. *LAV-BPIFB4* may function as a resilience amplifier—buffering downstream consequences of progerin-induced stress and linking reduced senescence burden to improved vascular integrity and diastolic function. This complementary framework is intended to generate testable biological hypotheses while remaining fully consistent with the original experimental observations.

Dispersion analysis and identification of candidate resilience nodes

Visual inspection of graphical distributions revealed heterogeneous responses across treated animals, with extreme data points clustering within functional and structural endpoints. Direct interrogation of tabulated numerical data further suggested that these extremes may reflect structured biological variability rather than artefacts of visualization.

The dialogue pinpointed that BPIFB4 expression increased consistently following treatment, indicating effective target engagement with relatively narrow dispersion compared with structural and functional variables. Notably, however, improvements in function and tissue remodelling did not scale linearly with BPIFB4 expression levels, suggesting that the modulation of BPIFB4 expression may function primarily as a permissive trigger rather than a quantitative driver. When examining the dispersion of the other validated data, the dialogue helped us to identify four specific nodes, as also illustrated in **Figure 3**:

- *Nuclear stress–adaptation senescence gating node.* Dispersion in baseline p21/p16 levels suggested heterogeneity in nuclear stress burden and stress-response activation. The animals exhibiting greater senescence signalling appeared to derive larger therapeutic benefit.

- *Vascular remodelling and arteriole adaptation node.* Variability in arteriole density and vascular pericyte coverage paralleled a reduction in perivascular fibrosis.

- *Extracellular matrix stiffness node.* Animals with higher baseline perivascular fibrosis appeared to exhibit disproportionately large improvements.

- *Diastolic performance threshold node.* Functional improvement appeared to emerge when fibrosis and senescence markers crossed specific ranges.

Taken together, the identified nodes suggest a reorganized hierarchical framework in which cardiovascular protection emerges from coordinated interactions between nuclear stress adaptation and vascular structural remodelling rather than from a single linear molecular cascade.

Discussion

This study illustrates the feasibility and informative value of using a structured analytical dialogue to extend interpretation of a previously published preclinical dataset from conceptual perspectives that were not emphasized in the original hypothesis-driven analysis. Rather than generating new biological evidence, the analytical process provided a transparent framework through which investigators re-examined validated experimental observations and developed complementary hypotheses for future investigation.

Applied to our previously published dataset, the structured analytical dialogue reaffirmed the primary biological signal associated with the intervention while suggesting a complementary hierarchical framework in which nuclear stress adaptation and vascular resilience may represent interacting regulatory processes associated with preservation of left ventricular diastolic function in progeria mice treated with a longevity-associated gene.

Our previous studies in non-progeric models of cardiomyopathy provide convergent evidence that *LAV-BPIFB4* gene therapy exerts both direct myocardial effects and broader vascular and stress-adaptive mechanisms. In type 2 diabetic mice, *LAV-BPIFB4* improved left ventricular relaxation, accompanied by adaptive reprogramming of contractile proteins.[19,20] Mechanistic insight was reinforced in human induced pluripotent stem cell-derived cardiomyocytes, where exposure to recombinant LAV-BPIFB4 protein improved contractile performance.[21] Additionally, delivery of the *LAV-BPIFB4* gene or recombinant protein rescued cardiac function and myocardial perfusion in diabetic and aged mice by improving microvascular density and pericyte coverage.[22] *LAV-BPIFB4*-primed pericytes from failing human hearts exhibited reduced expression of senescence markers, increased stress resilience, and enhanced proangiogenic activity through a mechanism involving the nucleolar protein nucleolin.[15] Taken together, these independent observations provide biological plausibility for the complementary interpretative framework generated in the present study, while remaining consistent with the original intervention-anchored mechanism. Importantly, they should not be regarded as independent validation of the hierarchical organization proposed through the structured analytical dialogue, which remains hypothesis-generating.

One contribution of the present analytical approach was to make explicit an interpretative process that investigators often perform implicitly when revisiting complex datasets. By systematically examining the published observations from alternative conceptual entry points while preserving the original experimental evidence, the structured analytical dialogue facilitated discussion of

biological relationships that were not emphasized in the original report. In this respect, the added value of the analytical process lies not in replacing hypothesis-driven interpretation, but in complementing it through a transparent and reproducible framework for generating biologically grounded hypotheses.

A second contribution of the structured analytical dialogue was the systematic examination of variability within the published dataset. Rather than focusing exclusively on group-average responses, investigators combined qualitative assessment of graphical dispersion with interrogation of individual-level numerical data to explore whether heterogeneous treatment responses could provide additional biological insight. This exploratory analysis suggested that therapeutic responsiveness was not uniformly distributed across treated animals and prompted consideration of whether biological context might influence the magnitude of phenotypic benefit despite consistent target engagement.

Within this analytical framework, the dialogue generated the hypothesis that *LAV-BPIFB4* may function less as a linear dose-to-effect driver and more as an initiator of adaptive biological processes whose downstream consequences depend on the pre-existing cellular and vascular context. Accordingly, the apparent heterogeneity in treatment response was interpreted as a source of biological information rather than experimental noise. Because these observations were derived from exploratory reinterpretation of a single published dataset, they should not be interpreted as evidence for discrete responder and non-responder populations but rather as candidate response trajectories requiring prospective validation.

During the structured analytical dialogue, investigators also reconsidered the observation that increased BPIFB4 immunoreactivity in treated animals could reflect not only transgene expression but also endogenous BPIFB4. The dialogue suggested that this possibility was biologically plausible, given the reported involvement of BPIFB4 in stress-adaptive signalling pathways. Although the original dataset cannot distinguish between endogenous and transgene-derived protein, this complementary interpretation generated the hypothesis that therapeutic gene delivery might initiate adaptive signalling capable of reinforcing endogenous BPIFB4 expression. This hypothesis provides one possible explanation for the absence of an obvious linear relationship between measured BPIFB4 protein levels and downstream functional improvement but remains to be experimentally tested.

From this perspective, the analytical dialogue further suggested that the principal therapeutic objective may not necessarily be maximal BPIFB4 expression but sufficient target engagement to activate adaptive pathways. This interpretation generated the hypothesis that future studies could investigate the minimal level of target engagement associated with functional benefit and evaluate whether biological markers of vascular remodelling or senescence burden identify conditions under which treatment is most effective. Such investigations may ultimately contribute to optimization of dose selection and integration with complementary therapeutic strategies, including farnesyltransferase inhibition or emerging gene-modifying approaches; however, implications remain speculative and extend beyond the evidence provided by the present study.

One practical outcome of the structured analytical dialogue was the development of an illustrative roadmap for future investigation. When investigators asked the AI platform to help prioritize subsequent experimental steps within realistic resource constraints, the dialogue suggested an initial focus on defining the target-engagement window, followed by evaluation of candidate biomarkers of fibrosis and senescence, selective integration with standard therapies, and validation of complementary mechanistic hypotheses in human-relevant experimental systems. This roadmap is presented as an example of how structured investigator-led analytical dialogue may assist in prioritizing future studies rather than as a prescriptive development strategy. More generally, this approach may complement established forms of post-publication scientific discussion, such as editorials and commentaries, by providing a structured framework for re-examining published findings from alternative conceptual perspectives.[22] This may be particularly valuable in rare genetic cardiovascular diseases, where small patient cohorts, limited availability of patient-derived material, and high experimental costs often restrict opportunities for experimental reiteration, making it especially important to maximize mechanistic insight from existing datasets.

An additional methodological consideration concerns our deliberate use of a general-purpose rather than a domain-adapted large language model. Domain-specific approaches, including fine-tuning on specialized biomedical corpora or integration of structured knowledge bases, may improve performance for narrowly defined analytical tasks. However, to our knowledge, no established domain-adapted AI platform has been specifically validated for HGPS datasets. Moreover, recent comparative studies indicate that such adaptation does not consistently outperform general-purpose language models on previously unseen biomedical reasoning tasks.[23,24] Moreover, in a different application, a recent study of 121 rare diseases reported that a general-purpose conversational model achieved the highest diagnostic accuracy among the evaluated systems without disease-

specific adaptation.[25] Although diagnostic reasoning differs from investigator-led reinterpretation of experimental datasets, these findings suggest that broad reasoning capability does not necessarily depend on domain-specific adaptation. Accordingly, our objective was not to maximize disease-specific knowledge retrieval, but to evaluate whether a widely accessible conversational AI could support structured analytical dialogue under continuous investigator supervision. We considered this approach to be more appropriate for assessing the feasibility and generalizability of the proposed methodology. Whether domain-specific adaptation would further enhance—or modify—the generation of complementary biological hypotheses in studies such as ours remains an open question that warrants direct comparative investigation.

Limitations

Several limitations should be acknowledged. First, this work represents a methodological proof-of-concept based on reinterpretation of a single previously published preclinical dataset. Consequently, the complementary conceptual framework generated through the structured analytical dialogue should not be regarded as independent biological validation of the proposed hierarchical organization**.** Dispersion-based interpretation retains an element of subjectivity despite integration with individual-level numerical analysis, and the proposed response trajectories, threshold-engagement model, and candidate resilience nodes remain analytical hypotheses requiring prospective evaluation in independent datasets.

Second, the analytical process relied on investigator-defined questions and continuous biological oversight. Although procedural safeguards—including predefined analytical boundaries, iterative evaluation, orthogonal conceptual exploration, and explicit acceptance criteria—were incorporated to reduce directional influence, alternative investigator groups might generate different complementary interpretations from the same dataset. This characteristic reflects the exploratory nature of structured analytical dialogue rather than a limitation unique to AI-assisted reasoning.

Finally, the present study was not designed to determine whether the proposed conceptual framework is biologically correct. Rather, its objective was to evaluate whether structured analytical dialogue could provide complementary perspectives that assist investigators in identifying hypotheses deserving future experimental investigation.

Conclusions and perspectives

This study illustrates the feasibility and informative value of incorporating structured analytical dialogue into investigator-led reinterpretation of experimental datasets. Rather than replacing hypothesis-driven investigation, the analytical process provided a transparent and reproducible framework through which validated observations could be re-examined from complementary conceptual perspectives while preserving the original experimental evidence.

Applied to a murine model of HGPS, the structured analytical dialogue generated complementary hypotheses suggesting that nuclear stress adaptation and vascular remodelling may represent interacting biological processes influencing therapeutic responsiveness following *LAV-BPIFB4* gene delivery. Future work should determine the reproducibility of this analytical strategy across independent datasets, biological systems, and investigator teams.

Acknowledgements

This work was funded by Italian Ministry of Health, Ricerca Corrente to the IRCCS MultiMedica. The authors used ChatGPT (GPT-5.5; OpenAI, San Francisco, CA, USA) as an investigator-supervised analytical support tool to facilitate structured scientific dialogue and explore alternative conceptual interpretations of previously published data. The authors take full responsibility for the content of this manuscript.

Competing Interests

A.A.P. has shares in LGV1 Inc. and has filed a patent. All other authors declare that they have no conflict of interest.

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Appendix

Structured Investigator–AI Analytical Dialogue Underlying Post-publication Reinterpretation

Model used: ChatGPT (GPT-5.5, OpenAI).
Interaction period: [February-June 2026].
Mode: Investigator-led iterative analytical dialogue.

1. Purpose of this Supplement

This Supplement has been prepared in response to the reviewers' request for greater transparency regarding the analytical process underlying the AI-assisted reinterpretation presented in the manuscript. Because the principal contribution of the study is methodological rather than computational, the investigator–AI dialogue itself constitutes an essential component of the scientific record.

The objective of this document is therefore to provide a transparent account of how the reinterpretation evolved, from the initial methodological questions through successive stages of biological analysis to the final conceptual framework incorporated into the revised manuscript. The Supplement illustrates the progression of analytical reasoning, the iterative refinement of ideas, and the continuous investigator oversight that governed acceptance, modification, or rejection of AI-assisted interpretations.

The dialogue should not be interpreted as a source of new experimental evidence. Rather, it shows how structured interaction between investigators and an AI platform was used to reorganize previously validated observations into complementary conceptual frameworks while remaining fully consistent with the original experimental findings.

2. Analytical Workflow Summary

The analytical dialogue was not conducted according to a predefined script but evolved iteratively as new biological and methodological questions emerged. Retrospective examination of the complete interaction nevertheless revealed a reproducible analytical workflow consisting of sequential reasoning cycles. This workflow progressively matured during the dialogue and subsequently guided revision of the manuscript.

Each analytical cycle consisted of five consecutive steps:

1. **Investigator-defined analytical question.** A biological or methodological question arising from critical re-examination of the published study was formulated by the investigators.
2. **AI-assisted analytical exploration.** The AI generated one or more conceptual interpretations or alternative biological organizations while remaining anchored to the information provided by the investigators and established scientific knowledge.
3. **Critical investigator evaluation.** Each response was examined for consistency with the published experimental observations, biological plausibility, and potential translational relevance.
4. **Iterative refinement.** Interpretations considered insufficiently supported, overly speculative, or inconsistent with the available evidence were modified, challenged through additional dialogue, or rejected.
5. **Conceptual synthesis.** Accepted concepts were incorporated into the evolving analytical framework and subsequently translated into the manuscript.

The dialogue evolved through seven broad analytical phases:

Phase	Principal objective
1	Define the methodological rationale for AI-assisted reinterpretation
2	Develop orthogonal analytical strategies complementary to the original intervention-anchored framework
3	Explore alternative biological hierarchies, including vascular remodelling and nuclear stress adaptation
4	Re-examine biological variability through dispersion analysis, responder heterogeneity, candidate resilience nodes, and threshold-like pathway engagement
5	Reinterpret BPIFB4 protein expression and distinguish target engagement from adaptive pathway activation
6	Reflect on investigator–AI interaction, analytical bias, and methodological implications
7	Translate the analytical dialogue into a scientifically rigorous manuscript while distinguishing observed findings from conceptual synthesis and future hypotheses

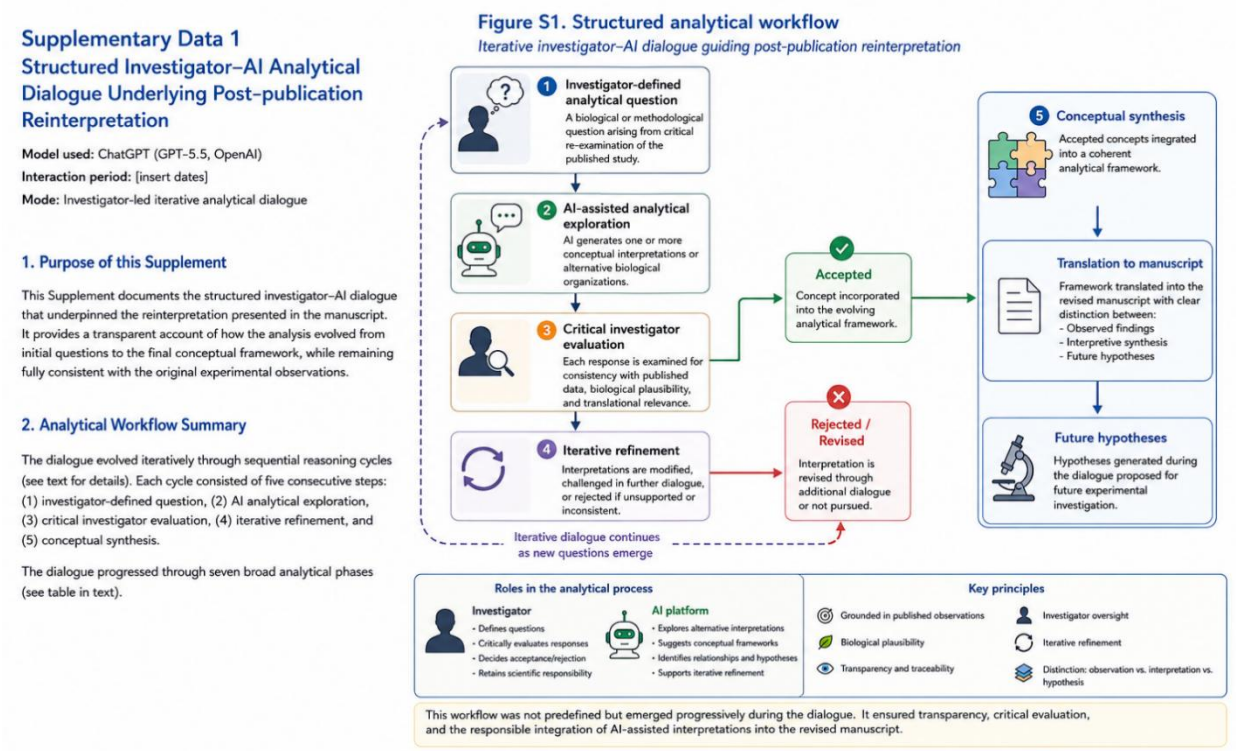
Throughout the dialogue, AI-generated interpretations were accepted only when they:

- remained fully compatible with the published experimental observations;
- did not contradict established biological knowledge;
- provided additional explanatory or translational value beyond the original interpretation; and
- could be clearly distinguished from experimentally demonstrated findings.

Scientific responsibility remained entirely with the investigators, who generated the analytical questions, critically evaluated every response, requested clarification or revision where necessary, and determined which concepts were incorporated into the manuscript.

How this Supplement should be read

The dialogue documents the evolution of scientific reasoning rather than a sequence of definitive conclusions. Early chapters illustrate exploratory conceptual development and comparison of alternative analytical pathways. Later chapters show how these ideas were progressively refined, moderated, integrated, or rejected before incorporation into the manuscript. Accordingly, some concepts appear in a broader or more exploratory form within the dialogue than in the final manuscript, reflecting the natural evolution of the analytical process.



3. Explanatory Note

The following chapters present a **faithfully reconstructed analytical transcript** derived from the complete investigator–AI conversation. The reconstruction was prepared directly from the original dialogue and is intended to preserve the scientific reasoning process while improving readability and facilitating independent evaluation of the analytical workflow.

To enhance clarity, the dialogue has been organized into thematic chapters that reflect the chronological evolution of the investigation. Within each chapter, the sequence of investigator questions and AI responses has been preserved as closely as possible.

Minor editorial revisions were limited to correction of typographical errors, removal of duplicated exchanges, consolidation of repetitive prompts, and omission of discussions exclusively related to language editing, formatting, reference management, or other editorial aspects not directly relevant to the scientific reasoning. Where brief linking sentences were required to preserve continuity following removal of repetitive material, these have been explicitly identified as editorial notes.

No scientific concepts, analytical steps, or investigator decisions have been intentionally omitted. The progression of reasoning, the evolution of alternative conceptual frameworks, and the continuous investigator oversight faithfully reflect the original dialogue.

The chapters that follow therefore document not only the conclusions ultimately incorporated into the manuscript but also the analytical pathway through which competing ideas were explored, critically evaluated, refined, or discarded before the final conceptual framework was established.

4. Glossary of analytical terminology

The following definitions are provided to facilitate interpretation of the analytical dialogue and the revised manuscript. These terms are used in a methodological rather than experimental sense and describe conceptual constructs that emerged during the investigator–AI dialogue. Unless explicitly supported by

experimental evidence, the terms should be interpreted as analytical frameworks or hypothesis-generating concepts rather than validated biological mechanisms.

Intervention-anchored framework

An interpretative framework in which the therapeutic intervention is considered the initiating event, and downstream molecular, structural, and functional changes are interpreted as consequences of successful pathway engagement.

Orthogonal analysis

Examination of the same dataset from a complementary conceptual entry point independent of the original hypothesis. This approach explores alternative biological organization while remaining compatible with the experimentally supported interpretation.

Resilience nodes

Interdependent biological processes proposed through AI-assisted reinterpretation to contribute to tissue adaptation and functional stability under stress. In the present study, nuclear stress adaptation and vascular remodelling are presented as **candidate** resilience nodes requiring prospective experimental validation.

Responder heterogeneity

Variation in treatment response among individuals within the same experimental group, interpreted as potentially reflecting biological context in addition to experimental variability. Within the present study, this concept is proposed as a hypothesis-generating interpretation derived from exploratory analysis of data dispersion.

Structured investigator–AI dialogue

An investigator-led iterative analytical process in which successive scientific questions, AI-assisted conceptual interpretations, critical biological evaluation, and repeated refinement are used to explore complementary organization of an existing dataset in a transparent and reproducible manner.

Threshold engagement model

A hypothesis-generating framework proposing that therapeutic benefit may emerge once sufficient biological pathway engagement has been achieved rather than increasing linearly with the magnitude of target expression.

Vascular–nuclear resilience perspective

An interpretative perspective in which vascular remodelling and nuclear stress adaptation are considered candidate upstream organizational layers contributing to preservation of cardiac function while maintaining the experimentally validated intervention-anchored interpretation.

Vertical reasoning

Traditional hypothesis-driven reasoning that progresses from hypothesis formulation through experimental testing toward mechanistic validation using targeted biological perturbation.

Chapter 1. Origin of the analytical dialogue

Defining the methodological question

Investigator

The purpose of this dialogue is not to generate new experimental data but to determine whether artificial intelligence can help reinterpret an already published preclinical study.

The original work had established a clear intervention–outcome relationship supported by experimental evidence. However, publication does not necessarily exhaust the scientific value of a dataset. During translational development, investigators frequently return to published results to generate new hypotheses, reassess mechanistic priorities, identify biomarkers, refine therapeutic strategies, and design subsequent experiments.

Can AI assist this post-publication analytical process in a structured and transparent way?

AI

Yes.

The informative life of an experimental dataset generally extends well beyond publication. Published results continue to influence scientific reasoning throughout the translational pathway by supporting mechanistic refinement, guiding experimental design, prioritizing biomarkers, informing dose optimization, and identifying opportunities for clinical development.

Traditionally, this post-publication reinterpretation relies almost entirely on investigator experience and iterative discussion within research groups.

Artificial intelligence may contribute differently.

Rather than replacing scientific expertise or generating new biological evidence, AI can function as a structured reasoning interface capable of systematically exploring alternative conceptual organizations while remaining anchored to the validated experimental observations.

In this context, the objective is not discovery of new data but expansion of the conceptual space available for interpreting existing evidence.

Investigator

This distinction is important.

The manuscript should therefore avoid presenting AI as a hypothesis generator or autonomous decision-maker.

Instead, AI should function as a reflective analytical companion supporting investigator-led reasoning.

AI

Agreed.

The analytical workflow should preserve a clear hierarchy of responsibilities.

The investigator defines the biological problem, formulates the analytical questions, evaluates biological plausibility, accepts or rejects proposed interpretations, and remains fully responsible for the scientific conclusions.

The AI contributes by proposing alternative conceptual organizations, highlighting relationships that may not be immediately apparent, and stimulating critical examination of established interpretative frameworks.

Scientific authority therefore remains entirely with the investigator.

Why select a published study in Hutchinson–Gilford Progeria Syndrome?

Investigator

If this methodology is to be evaluated, the choice of dataset becomes important.

Why is our published BPIFB4 study in Hutchinson–Gilford Progeria Syndrome an appropriate model?

AI

Several characteristics make this study particularly suitable.

First, the original publication already established a coherent biological narrative supported by experimental evidence. This provides a stable evidentiary baseline against which reinterpretation can be assessed.

Second, the intervention improved cardiovascular phenotype without modifying progerin accumulation. This creates an opportunity to explore whether downstream adaptive mechanisms may contribute to therapeutic benefit despite persistence of the primary genetic defect.

Third, rare diseases inherently generate relatively small datasets, limiting opportunities for repeated experimentation. Consequently, maximizing the analytical value of existing data has particular translational importance.

Finally, the study integrates molecular, structural, vascular, and functional endpoints, allowing alternative biological hierarchies to be explored without introducing new experimental observations.

Investigator

Therefore, the purpose is not to question the original publication.

Rather, the objective is to determine whether the same validated observations can support complementary conceptual frameworks capable of extending translational interpretation.

AI

Correct.

The analytical dialogue should preserve three fundamental principles throughout:

1. Experimental observations remain unchanged.
2. Original biological conclusions remain valid.
3. Alternative interpretations must remain compatible with both the published evidence and established biological knowledge.

The dialogue therefore seeks conceptual expansion rather than reinterpretation through contradiction.

Transition toward analytical exploration

Investigator

How should the analytical process begin?

AI

The dialogue should first establish the conventional intervention-anchored interpretation of the published study.

Only after defining this reference framework should alternative conceptual organizations be explored.

This sequence provides an explicit comparator throughout the analysis and prevents reinterpretation from becoming detached from the original experimental evidence.

Consequently, the next analytical stage should examine whether the validated observations can be reorganized through orthogonal conceptual entry points while preserving their experimental meaning.

Editorial note

The analytical considerations developed in this opening dialogue formed the basis for the methodological framework described in the Introduction and Methods sections of the revised manuscript. They established the central premise that structured human–AI dialogue was intended to expand conceptual interpretation while preserving the integrity of the original experimental evidence.

Chapter 2. From conventional hypothesis-driven interpretation to orthogonal analytical reasoning

Introducing an alternative analytical perspective

Investigator

Biological research is usually conducted through a vertical reasoning process. Investigators formulate a hypothesis, design experiments to test cause-and-effect relationships, and subsequently validate the proposed mechanism using genetic or pharmacological perturbation.

Can an already published dataset be examined from a different conceptual entry point without challenging the original conclusions?

AI

Yes.

Conventional biological reasoning follows a predominantly vertical structure.

Theory generates hypotheses.

Hypotheses guide experiments.

Experimental observations establish causal relationships.

Mechanistic studies then strengthen or refute the proposed pathway.

This strategy is fundamental for demonstrating biological causality.

However, once a dataset has been generated and experimentally validated, the same observations may still permit alternative conceptual organizations.

Rather than testing a new hypothesis experimentally, investigators may ask whether the validated findings can be reorganized into complementary mechanistic hierarchies that were not emphasized during the original interpretation.

This represents an orthogonal analytical perspective rather than a competing biological theory.

Investigator

Therefore, orthogonal reasoning should not replace conventional hypothesis-driven science.

AI

Correct.

The purpose of orthogonal reasoning is complementary rather than competitive.

It preserves the experimental evidence while exploring alternative biological entry points.

Consequently, the dialogue should remain firmly anchored to the published observations.

Every reinterpretation should satisfy three conditions:

- remain compatible with the experimental data;
- remain biologically plausible;
- provide additional explanatory or translational value.

Can multiple hierarchies coexist?

Investigator

If several conceptual organizations are biologically plausible, should one be considered correct and the others incorrect?

AI

Not necessarily.

Complex biological systems frequently permit multiple hierarchical organizations.

Experimental observations establish what occurred.

Hierarchy determines how these observations are conceptually organized.

Different hierarchies may therefore coexist without contradicting one another, provided that they remain compatible with the underlying evidence.

Consequently, reinterpretation concerns organization rather than replacement of biological mechanisms.

Investigator

Would this distinction help explain why different experts often interpret the same paper differently?

AI

Yes.

Investigators usually agree on the experimental observations.

Differences more commonly arise in the biological hierarchy assigned to those observations.

Experience, disciplinary background, and prevailing conceptual models influence which variables are considered primary and which are viewed as downstream consequences.

Structured dialogue allows these implicit hierarchies to become explicit and therefore open to critical evaluation.

Can AI contribute to this process?

Investigator

Many current applications of artificial intelligence focus on prediction, classification, or image analysis.

What role could AI play in this analytical setting?

AI

The role differs fundamentally.

AI is not being asked to predict experimental outcomes or generate new biological evidence.

Instead, it functions as a structured reasoning interface.

Its contribution consists of exploring alternative conceptual organizations that remain compatible with validated observations.

The objective is to widen the analytical search space while allowing the investigator to determine which interpretations remain biologically credible.

Accordingly, the dialogue becomes an iterative reasoning exercise rather than an automated discovery process.

Investigator

Can AI itself determine which interpretation is biologically correct?

AI

No.

The AI has no independent access to experimental truth.

Its responses are evaluated entirely by the investigator.

Interpretations inconsistent with published observations or established biological knowledge should be rejected.

Consequently, biological validity remains under investigator control throughout the analytical process.

Choosing the analytical entry points

Investigator

If we are going to reinterpret the published BPIFB4 study systematically, should we define the conceptual pathways before beginning the dialogue?

AI

Yes.

Predefining broad analytical domains improves transparency and reduces the risk of post hoc interpretation.

Rather than exploring unlimited possibilities, the dialogue can be organized around a small number of complementary conceptual trajectories.

Each trajectory should represent a distinct biological perspective while remaining compatible with the original experimental observations.

Investigator

Which trajectories appear most appropriate for the present study?

AI

Three analytical domains appear particularly suitable.

1. Intervention-anchored framework

The original biological interpretation, beginning with BPIFB4 delivery and following the established sequence of downstream molecular, structural and functional effects.

2. Nuclear stress pathway

Exploring whether adaptation to persistent nuclear stress may organize the observed reduction in senescence despite unchanged progerin.

3. Vascular-first systems pathway

Examining whether preservation of vascular architecture provides an upstream organizational layer capable of integrating extracellular matrix remodeling and ventricular function.

These pathways should initially be explored independently before assessing whether they converge into a unified conceptual framework.

Investigator

Therefore, the objective is not to identify the "correct" hierarchy but to determine whether complementary organizations emerge from the same experimental observations.

AI

Exactly.

The dialogue should preserve the original intervention-anchored interpretation while evaluating whether additional organizational structures become apparent through systematic orthogonal reasoning.

The analytical process therefore proceeds by comparison rather than replacement.

Editorial note

The concepts developed during this phase of the dialogue established the methodological basis for the investigator-defined analytical prompts subsequently incorporated into the structured workflow. They also motivated the organization of reinterpretation into predefined conceptual domains, which later evolved into the nuclear stress, vascular resilience, and translational analytical frameworks described in the manuscript.

Chapter 3. Exploring alternative biological organizations of the BPIFB4–HGPS dataset

Searching for complementary mechanistic frameworks

Investigator

The original publication interpreted the biological effects of LAV-BPIFB4 through a conventional intervention-anchored framework: gene delivery increased BPIFB4 expression, leading to structural and functional improvements. Can the same observations be reorganized into alternative biological hierarchies without contradicting the published conclusions?

AI

Yes.

The intervention-anchored framework remains entirely valid because it reflects the experimental design.

However, the same observations can also be organized from different biological entry points.

Rather than asking **"What does BPIFB4 do?"**, one may ask **"What biological processes best organize the observed phenotype?"**

This shifts attention from the intervention itself toward the adaptive responses that ultimately determine cardiac function.

Several complementary organizational pathways appear plausible.

First exploratory pathway: autophagy and TFEB

Investigator

Since progerin is known to impair proteostasis, could autophagy represent the principal organizing mechanism?

AI

This is biologically plausible.

Progerin accumulation disrupts nuclear architecture and impairs nucleocytoplasmic trafficking. Experimental studies have reported reduced activity of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, autophagy, and cellular quality-control mechanisms.

Restoration of TFEB-dependent pathways could theoretically reduce cellular stress, improve proteostasis, and attenuate senescence despite persistence of progerin.

However, the published BPIFB4 dataset contains no measurements of TFEB activity, autophagic flux, or lysosomal function.

Consequently, although biologically attractive, this pathway would require substantial extrapolation beyond the available evidence.

Investigator

Would this become the central interpretation?

AI

Probably not.

It represents a plausible mechanistic hypothesis but is weakly anchored to the measured variables.

It is better considered as a possible downstream explanatory pathway rather than the principal organizational framework.

Investigator decision

The TFEB/autophagy pathway was retained as a mechanistic hypothesis but not selected as the primary hierarchical organization.

Second exploratory pathway: vascular remodeling

Investigator

Could vascular remodeling provide a stronger organizing principle?

AI

The published observations support this possibility more directly.

The dataset documents increased coronary arteriole density together with reduced perivascular fibrosis and preserved ventricular relaxation.

Unlike TFEB, these variables were measured directly.

Microvascular integrity influences myocardial perfusion, extracellular matrix homeostasis, oxygen delivery, and ventricular compliance.

Consequently, vascular remodeling represents a biologically measurable adaptive process linking molecular changes to organ function.

Investigator

Would this interpretation contradict the original paper?

AI

No.

The intervention remains the initiating event.

Only the hierarchical emphasis changes.

Instead of considering vascular remodeling simply as a downstream consequence of BPIFB4 delivery, it becomes a major adaptive layer through which functional preservation may emerge.

Investigator decision

The vascular pathway was selected for further exploration.

Third exploratory pathway: nuclear stress adaptation

Investigator

One finding continues to intrigue me.

Progerin levels remained unchanged, yet senescence markers were reduced.

Could adaptation to nuclear stress represent another organizing principle?

AI

This possibility appears particularly interesting.

If progerin accumulation persists, the reduction in p21 and related senescence markers cannot reasonably be attributed to correction of the primary genetic defect.

Instead, the observations suggest increased tolerance of persistent nuclear injury.

The intervention therefore appears to buffer the downstream consequences of progerin rather than eliminating its cause.

This shifts the interpretation from mutation correction toward enhancement of cellular resilience.

Investigator

How does this differ from the vascular interpretation?

AI

The vascular pathway begins at tissue organization.

The nuclear stress pathway begins at the cellular response to persistent nuclear injury.

They therefore represent different analytical entry points rather than competing biological mechanisms.

One starts with the vasculature.

The other starts with cellular adaptation.

Both converge toward preservation of myocardial structure and function.

Comparing the candidate pathways

Investigator

Can we compare the relative strengths of these different interpretations?

AI

Yes.

Each pathway may be evaluated according to three criteria.

1. Direct support from the published dataset

- Intervention-anchored framework: very strong.
- Vascular remodeling: strong.
- Nuclear stress adaptation: moderate.
- TFEB/autophagy: indirect.

2. Biological plausibility

All four pathways are biologically plausible and consistent with current knowledge.

3. Translational value

The vascular and nuclear stress pathways appear particularly valuable because they integrate multiple measured variables and generate experimentally testable hypotheses for future work.

Investigator

Should only one framework be retained?

AI

Not necessarily.

The strongest interpretation may emerge from combining complementary pathways into a hierarchical network.

Rather than selecting one dominant mechanism, the dialogue suggests that intervention-anchored reasoning, vascular remodeling, and nuclear stress adaptation represent different levels of biological organization.

Investigator decision

The analytical strategy shifted from identifying a single alternative mechanism toward integrating complementary hierarchical frameworks.

This transition marked an important conceptual turning point in the dialogue.

Emergence of systems-level integration

Investigator

Instead of choosing between these pathways, could they represent different layers within the same adaptive network?

AI

Yes.

This appears to be the most coherent interpretation.

BPIFB4 delivery remains the initiating intervention.

Nuclear stress adaptation represents the cellular response.

Vascular remodeling represents the tissue-level adaptation.

Extracellular matrix remodeling links vascular integrity to myocardial mechanics.

These adaptive layers converge on preservation of ventricular relaxation.

Rather than replacing the original intervention-anchored interpretation, this systems-level organization expands it by introducing hierarchical interactions among experimentally observed variables.

Investigator

This appears closer to systems biology than to classical linear causality.

AI

Correct.

The dialogue has progressively shifted from a linear intervention–effect sequence toward a hierarchical adaptive network.

Importantly, this transition emerged from repeated comparison of alternative organizational pathways rather than from introduction of new experimental evidence.

Editorial note

This stage of the dialogue represented a major conceptual transition.

Instead of searching for a single alternative mechanism, the analytical process progressively converged on the idea that multiple biologically plausible entry points could coexist within a common hierarchical framework.

This reasoning subsequently informed the development of Figures 1 and 2, the Results section describing complementary conceptual trajectories, and the Discussion emphasizing interdependent nuclear and vascular adaptive layers.

Chapter 4. From average treatment effects to biological heterogeneity

Re-examining the information contained within the dataset

Investigator

The original publication interpreted the study using conventional group comparisons based on mean values and statistical significance. However, while reviewing the figures, I was struck by the apparent heterogeneity among individual animals. Could this variability itself contain biologically meaningful information?

AI

Potentially yes.

Group averages summarize the dominant biological effect and remain essential for demonstrating treatment efficacy. However, they also compress the variability of individual responses.

Visual inspection of individual datapoints can sometimes reveal patterns that are not apparent from summary statistics alone.

Rather than asking only whether treatment changes the mean response, one may ask why animals receiving the same intervention exhibit different degrees of structural or functional improvement.

This shifts the analytical focus from average efficacy toward biological responsiveness.

Investigator

Would this constitute a new statistical analysis?

AI

No.

The published statistical analysis remains unchanged.

Inspection of dispersion represents an exploratory analytical exercise intended to generate biological hypotheses rather than confirm them statistically.

Consequently, any interpretation arising from variability must be presented explicitly as hypothesis-generating.

Exploring structured variability

Investigator

Can the apparent dispersion simply reflect random experimental noise?

AI

That possibility cannot be excluded.

However, when technical artefacts have been minimized through appropriate experimental procedures, biological variability itself may become informative.

The important question is not whether variability exists—it invariably does—but whether the pattern of variability appears compatible with underlying biological organization.

The present dataset cannot establish this formally.

Nevertheless, the distribution of several variables appears sufficiently structured to justify further analytical exploration.

Investigator

Should we therefore regard the extreme datapoints as biologically important?

AI

Not automatically.

Extreme observations should not be interpreted as evidence of responder subgroups without independent validation.

However, they may identify biological conditions worthy of further investigation.

Accordingly, the dialogue should distinguish clearly between observation, interpretation, and hypothesis.

Relationship between target engagement and biological response

Investigator

One aspect particularly attracted my attention.

BPIFB4 expression appeared to increase consistently in treated animals, whereas fibrosis, arteriole density, and diastolic function exhibited considerably greater variability.

Could this discrepancy itself be biologically informative?

AI

Yes.

This comparison suggests that successful target engagement alone may not fully determine therapeutic outcome.

If expression changes relatively uniformly whereas downstream phenotypes remain heterogeneous, additional biological factors are likely to influence treatment response.

Consequently, BPIFB4 expression may function primarily as an initiating event, whereas the magnitude of benefit depends upon the adaptive capacity of the recipient tissue.

This interpretation remains entirely compatible with the published data while providing an alternative perspective on therapeutic responsiveness.

Investigator

Does this imply that expression is unimportant?

AI

No.

Effective expression remains necessary.

However, once adequate biological activity has been achieved, downstream adaptive processes may become the principal determinants of phenotypic variability.

Thus, target engagement and therapeutic response should not necessarily be viewed as quantitatively equivalent.

Emergence of candidate resilience nodes

Investigator

Can these heterogeneous responses be organized into broader biological categories?

AI

Rather than considering each variable independently, the observations appear to cluster into several interacting biological domains.

These domains may be regarded as candidate resilience nodes because each represents an adaptive process capable of influencing overall cardiovascular outcome.

The principal candidate nodes emerging from the dialogue are:

Nuclear stress adaptation

reflecting variability in senescence-associated signalling.

Microvascular structural adaptation

reflecting arteriole density and vascular organization.

Extracellular matrix remodelling

reflecting perivascular fibrosis and myocardial stiffness.

Functional adaptation

represented by preservation of ventricular relaxation.

These should be regarded as conceptual organizational units rather than experimentally validated mechanistic entities.

Investigator

Could these nodes explain why animals respond differently despite similar treatment?

AI

They provide one possible conceptual explanation.

If therapeutic benefit depends upon the interaction among several adaptive processes rather than a single molecular event, biological variability would be expected even under comparable levels of target engagement.

This interpretation naturally introduces the concept of biological resilience.

However, the present dataset is insufficient to establish causal relationships among these nodes.

Future studies would be required to determine their relative hierarchy.

Emergence of threshold thinking

Investigator

During our discussion, I have the impression that the response does not increase continuously with expression.

Rather, it seems to appear once a certain biological condition has been achieved.

AI

That impression is compatible with the observed patterns.

Instead of a continuously linear relationship, one may hypothesize that adaptive pathways require sufficient biological engagement before measurable tissue benefit becomes evident.

Beyond this point, additional increases in expression might produce progressively smaller incremental effects because downstream adaptive capacity becomes limiting.

Importantly, this represents a conceptual hypothesis rather than a demonstrated biological threshold.

Investigator

Would this interpretation have translational implications?

AI

Potentially.

If confirmed experimentally, future dose-optimization studies might focus on achieving reliable pathway engagement rather than maximal transgene expression.

However, the present study neither measures dose-response relationships nor validates such thresholds.

Accordingly, this implication should be presented cautiously as a possible consequence of the analytical reinterpretation.

Integrating heterogeneity into the analytical framework

Investigator

Our discussion has progressively moved away from average treatment effects toward variability itself.

Does this change the overall analytical philosophy?

AI

Yes.

The dialogue has evolved from asking whether BPIFB4 improves cardiac phenotype to asking why improvement varies among apparently similar animals.

This shift does not alter the published conclusions regarding efficacy.

Instead, it introduces biological heterogeneity as an additional analytical dimension.

Within this framework, variability is no longer viewed solely as statistical dispersion but also as a potential source of mechanistic insight.

Investigator

I think this represents one of the principal conceptual advances emerging from the dialogue.

AI

I agree.

The reinterpretation does not generate new biological observations.

Rather, it extracts additional conceptual information from existing observations by treating biological heterogeneity as an analytical opportunity rather than merely experimental noise.

Editorial note

This stage of the dialogue represented the transition from conventional interpretation based on average treatment effects to a systems-oriented perspective incorporating biological heterogeneity. The concepts of candidate resilience nodes, pathway engagement, threshold-like behaviour, and responder variability subsequently became central elements of the revised Results and Discussion. Throughout manuscript revision these concepts were consistently presented as analytical hypotheses requiring prospective experimental validation rather than as experimentally demonstrated mechanisms.

Chapter 5. Reinterpreting BPIFB4 expression: from transgene abundance to pathway engagement

A methodological observation revisited

Investigator

While revising the manuscript, I realized that one methodological aspect deserves further consideration.

In the original publication, cardiac BPIFB4 protein expression was assessed using an antibody that recognizes both endogenous murine BPIFB4 and the human BPIFB4 transgene. Consequently, the increase in signal observed after treatment cannot be interpreted as reflecting only expression of the transferred gene.

This limitation was already acknowledged in the original publication.

However, what could be the biological implications of this observation for reinterpreting the dataset?

AI

This question shifts the discussion from analytical methodology to biological interpretation.

The important point is not simply that the antibody lacks species specificity.

Rather, the measured BPIFB4 signal may represent two biologically distinct processes occurring simultaneously:

- successful expression of the therapeutic transgene;
- modulation of endogenous BPIFB4 protein within the recipient myocardium.

Accordingly, the measured increase in BPIFB4 should not necessarily be interpreted solely as a quantitative marker of gene transfer.

Instead, it may represent the overall biological response of the BPIFB4 pathway.

Investigator

This distinction was not considered in the original publication.

Would this change the biological interpretation?

AI

The original conclusions remain unchanged.

However, the biological meaning assigned to BPIFB4 expression becomes broader.

Instead of representing only transgene abundance, the measured protein level may indicate overall pathway engagement resulting from interaction between exogenous gene delivery and endogenous adaptive responses.

Consequently, expression becomes a marker of biological activation rather than simply vector performance.

Linking pathway engagement to biological variability

Investigator

This interpretation appears particularly interesting when considered together with the heterogeneous structural responses observed among treated animals.

Could these two observations be related?

AI

Potentially yes.

If BPIFB4 expression reflects successful activation of a biological programme rather than simply the amount of transferred gene, one would not necessarily expect a linear relationship between protein abundance and downstream phenotypic improvement.

Instead, once sufficient pathway engagement has been achieved, subsequent differences in therapeutic response may depend predominantly upon the biological condition of the recipient tissue.

Under this interpretation, variation in fibrosis regression, vascular remodelling, or ventricular relaxation reflects differences in tissue responsiveness rather than differences in transgene expression.

Investigator

Would this imply that expression is no longer important?

AI

No.

Effective expression remains essential because it initiates pathway activation.

The reinterpretation simply suggests that, after successful engagement, downstream adaptive mechanisms become progressively more important in determining the final phenotype.

Thus, target engagement and biological responsiveness represent related but distinct concepts.

Considering endogenous BPIFB4 induction

Investigator

If endogenous BPIFB4 contributes to the measured signal, could gene delivery itself stimulate native BPIFB4 expression?

AI

This possibility is biologically plausible.

The observed increase in total BPIFB4 protein may reflect not only production of the human transgene but also activation of endogenous regulatory mechanisms.

Such amplification would be consistent with a feed-forward adaptive response in which the therapeutic intervention enhances intrinsic resilience pathways.

However, the present dataset cannot experimentally distinguish endogenous induction from transgene-derived protein.

Consequently, this possibility should be presented explicitly as a hypothesis requiring validation using species-specific analytical methods.

Investigator

Therefore, the novelty lies not in the antibody itself, but in asking a different question.

AI

Exactly.

The methodological limitation was already recognized.

The analytical innovation arises from exploring its biological implications.

Implications for translational development

Investigator

Could this reinterpretation influence future therapeutic development?

AI

Potentially.

If biological pathway engagement proves more informative than transgene abundance alone, future translational studies may benefit from distinguishing:

- vector delivery,
- pathway activation,
- downstream adaptive response,

rather than considering them equivalent measures of therapeutic success.

This perspective may also encourage development of biomarkers reflecting biological pathway activation instead of relying exclusively on transgene expression.

Nevertheless, these implications remain conceptual and require prospective validation.

Investigator

Would you therefore recommend modifying the Discussion rather than the Results?

AI

Yes.

The experimental observations themselves remain unchanged.

The reinterpretation concerns their biological implications.

Consequently, these concepts are more appropriately discussed within the Discussion, where they can be clearly distinguished from experimentally demonstrated findings.

Emergence of a broader conceptual principle

Investigator

It seems that this discussion extends beyond BPIFB4.

AI

I agree.

The dialogue suggests a more general conceptual distinction applicable to many biological interventions.

Successful therapeutic delivery should not automatically be equated with biological responsiveness.

Rather, effective treatment may involve sequential levels of organisation:

Therapeutic intervention

↓

Target engagement

↓

Activation of adaptive biological pathways

↓

Structural remodelling

↓

Functional improvement

The BPIFB4 dataset provides one example in which this distinction emerged through structured reinterpretation.

Future studies will determine whether this organisational principle has broader applicability.

Editorial note

This dialogue represented one of the clearest examples of AI-assisted conceptual reinterpretation during the project. The methodological observation regarding antibody specificity was already known and reported in the original publication. The novelty emerged from asking a new biological question concerning the implications of that observation for interpreting therapeutic response. The resulting distinction between target engagement and adaptive pathway activation subsequently influenced the revised Discussion, translational perspectives, and future research roadmap, while remaining fully consistent with the published experimental findings.

Chapter 6. Reflection on AI-assisted scientific reasoning: from biological interpretation to methodological framework

Does AI actually "reason"?

Investigator

Throughout this dialogue I have the impression that AI is not simply retrieving information but reorganizing concepts.

Can this process legitimately be described as reasoning?

AI

The term "reasoning" should be used cautiously.

The AI does not reason in the human cognitive sense, nor does it possess scientific intuition or independent understanding of biological systems.

However, it can generate coherent analytical structures by integrating information provided during the dialogue with established scientific knowledge.

Within this project, the important point is not whether AI reasons exactly as humans do, but whether the dialogue supports investigator-led analytical exploration.

The scientific reasoning therefore emerges from the interaction rather than from either participant independently.

Investigator

So the unit of analysis becomes the dialogue itself rather than the AI.

AI

Exactly.

Neither the investigator nor the AI alone produced the final conceptual framework.

The framework progressively emerged through iterative questioning, critical evaluation, reformulation, and biological validation.

The dialogue therefore represents the analytical process.

Human and AI biases

Investigator

Humans have conceptual biases because we tend to interpret data according to our previous hypotheses.

Does AI exhibit different biases?

AI

Yes.

The sources of bias differ.

Human investigators often show:

- confirmation bias,
- attachment to previous hypotheses,
- disciplinary preferences,
- translational expectations.

AI, in contrast, may exhibit:

- preference for internally coherent explanations,
- tendency to integrate rather than discriminate,
- excessive confidence if not challenged,
- dependence upon the framing of the prompt.

Neither source of bias is inherently superior.

The analytical advantage arises when each compensates for the limitations of the other.

Investigator

Therefore the dialogue itself becomes a mechanism for exposing hidden assumptions.

AI

Precisely.

Repeated questioning forces both participants to justify conceptual choices.

Alternative interpretations become explicit instead of remaining implicit.

This transparency represents one of the principal methodological strengths of structured dialogue.

Hidden conceptual forks

Investigator

During our discussions I repeatedly noticed that the dialogue generated alternative conceptual pathways that had not initially occurred to me.

Could these represent hidden conceptual forks?

AI

Yes.

Scientific interpretation often follows the first biologically plausible hierarchy identified by investigators.

However, multiple compatible organizations may exist.

Structured dialogue encourages exploration of these latent alternatives before selecting the framework ultimately incorporated into the manuscript.

Importantly, many of these alternative paths are subsequently rejected.

Nevertheless, documenting their existence makes the analytical process more transparent.

Investigator

Therefore disagreement becomes informative.

AI

Yes.

Moments of disagreement frequently identify conceptual decision points.

Rather than representing failure of the dialogue, these forks often stimulate deeper analysis and lead to more robust final interpretations.

When should AI be challenged?

Investigator

Several times I asked you to moderate your conclusions because they appeared stronger than the available evidence.

Should this be considered an essential component of the methodology?

AI

Absolutely.

The dialogue should not consist of passive acceptance of AI outputs.

Investigator criticism constitutes an essential validation step.

Throughout this project numerous responses were modified after concerns regarding:

- excessive mechanistic certainty,
- overstatement of causality,
- unsupported translational implications,
- inappropriate terminology,
- distinction between observation and hypothesis.

These revisions illustrate investigator control rather than AI autonomy.

Emergence of the methodological contribution

Investigator

Initially I considered this a biological reinterpretation.

It now appears increasingly to be a methodological study.

AI

The dialogue evolved in exactly that direction.

The biological reinterpretation provided the case study.

The principal contribution gradually became the analytical methodology itself.

The study therefore shifted from asking:

"Can BPIFB4 be interpreted differently?"

toward:

"Can structured human–AI dialogue become a transparent analytical methodology for post-publication reinterpretation?"

Investigator

This was not the original objective.

AI

Correct.

The methodological contribution emerged progressively during the dialogue itself.

This illustrates one of the distinctive characteristics of exploratory analytical interaction: the objective may evolve as conceptual understanding develops.

Defining the role of AI

Investigator

One concern is that readers may conclude that AI generated the science.

How should this misunderstanding be avoided?

AI

The dialogue demonstrates the opposite.

The investigator:

- selected the dataset,
- formulated every scientific question,
- challenged interpretations,
- rejected unsupported conclusions,
- decided what entered the manuscript.

The AI:

- proposed alternative conceptual organizations,
- generated comparative frameworks,
- identified relationships among observations,
- suggested additional hypotheses,
- supported iterative refinement.

Scientific responsibility therefore remained entirely with the investigators.

Editorial note

This phase of the dialogue marked an important transition in the project. The emphasis progressively shifted from reinterpreting a specific biological dataset to defining a general methodology for investigator-led analytical dialogue with artificial intelligence. Many concepts subsequently incorporated into the revised Introduction, Methods, Discussion, and Conclusion—including the distinction between observed findings,

conceptual synthesis, and future hypotheses—originated during this methodological reflection rather than from the biological reinterpretation itself.

Chapter 7. Translating analytical dialogue into a scientific manuscript

From exploratory reasoning to scientific reporting

Investigator

At this stage, the dialogue has generated several conceptual frameworks. However, not all of them can be incorporated into the manuscript.

How should we decide what remains?

AI

The manuscript should not attempt to reproduce the entire analytical exploration.

Instead, it should retain only those concepts that satisfy four criteria:

- they remain fully supported by the published observations;
- they provide additional explanatory value;
- they are biologically plausible;
- they are clearly distinguished from experimentally demonstrated findings.

Ideas that remain speculative should either be removed or explicitly presented as hypotheses for future investigation.

Investigator

Therefore, the manuscript should become more conservative than the dialogue itself.

AI

Yes.

Exploratory dialogue intentionally generates multiple conceptual possibilities.

Scientific reporting requires progressive refinement.

Consequently, the final manuscript should represent the most biologically robust subset of the analytical discussion rather than the complete conversation.

Moderating the language

Investigator

Several interpretations sound stronger than the available evidence.

For example:

- resilience nodes,
- threshold engagement,
- vascular-first hierarchy,
- nuclear stress adaptation.

How should these concepts be presented?

AI

Throughout the manuscript, terminology should distinguish between:

Observed findings

and

Interpretative synthesis.

Expressions implying experimental demonstration should be replaced by language indicating conceptual interpretation.

For example,

instead of

"BPIFB4 functions as a resilience amplifier"

one may write

"The AI-assisted reinterpretation suggests that BPIFB4 may function as a resilience amplifier."

Similarly,

instead of

"vascular remodeling precedes functional recovery"

one may write

"vascular remodeling emerges as a candidate upstream adaptive layer."

This maintains scientific caution while preserving the conceptual contribution.

Investigator

This distinction should probably be applied consistently throughout the manuscript.

AI

Agreed.

The same principle should govern the Abstract, Results, Discussion, Figures, and Conclusion.

Separating observation from interpretation

Investigator

Is important to distinguish between data-supported observations and AI-generated interpretation.

How can this be achieved?

AI

The manuscript should consistently follow three sequential levels.

Level 1

Published experimental observations.

Level 2

Analytical reinterpretation generated through structured dialogue.

Level 3

Future hypotheses requiring prospective validation.

Maintaining this structure throughout the manuscript prevents conceptual overstatement and improves methodological transparency.

Investigator

This also seems applicable to the figures.

AI

Yes.

Figures should visually distinguish:

original experimental observations,

interpretative overlays,

and future conceptual implications.

This separation reinforces the methodological nature of the study.

Looking back at the dialogue

Investigator

After completing this analytical journey, what do you consider the principal contribution?

AI

The dialogue did not generate new experimental observations.

Neither did it establish new biological mechanisms.

Its principal contribution was to demonstrate that structured interaction between investigators and artificial intelligence can make explicit an analytical process that is usually performed informally and remains undocumented.

By recording the evolution of scientific reasoning—from the original hypothesis, through alternative conceptual pathways, to the final integrated framework—the dialogue itself becomes part of the scientific record.

Investigator

That was not the original intention when we started.

AI

No.

The initial objective was to reinterpret a published dataset.

During the dialogue, however, the methodological implications progressively became more important than the specific biological example.

Consequently, the project evolved naturally into a methodological proof-of-concept using BPIFB4 in progeria as its illustrative case study.

Final reflection

Investigator

Do you think this supplementary dialogue should be viewed simply as supporting material?

AI

Not entirely.

Although it accompanies the manuscript, it also serves as an example of transparent scientific reasoning.

Traditionally, scientific papers report only the final interpretation.

This document instead records how competing ideas were explored, refined, rejected, integrated, and ultimately translated into the published manuscript.

In doing so, it illustrates a possible framework for documenting structured investigator–AI analytical dialogue in future studies.

Editorial note

This final phase completed the transition from exploratory scientific dialogue to formal scientific reporting. The manuscript that emerged represents a filtered synthesis of the broader analytical exploration, retaining only concepts judged by the investigators to be compatible with the published evidence and sufficiently robust for scientific communication. The complete dialogue presented in this Supplement therefore documents not only the conclusions ultimately incorporated into the manuscript but also the reasoning process through which those conclusions were critically examined, refined, and contextualized.

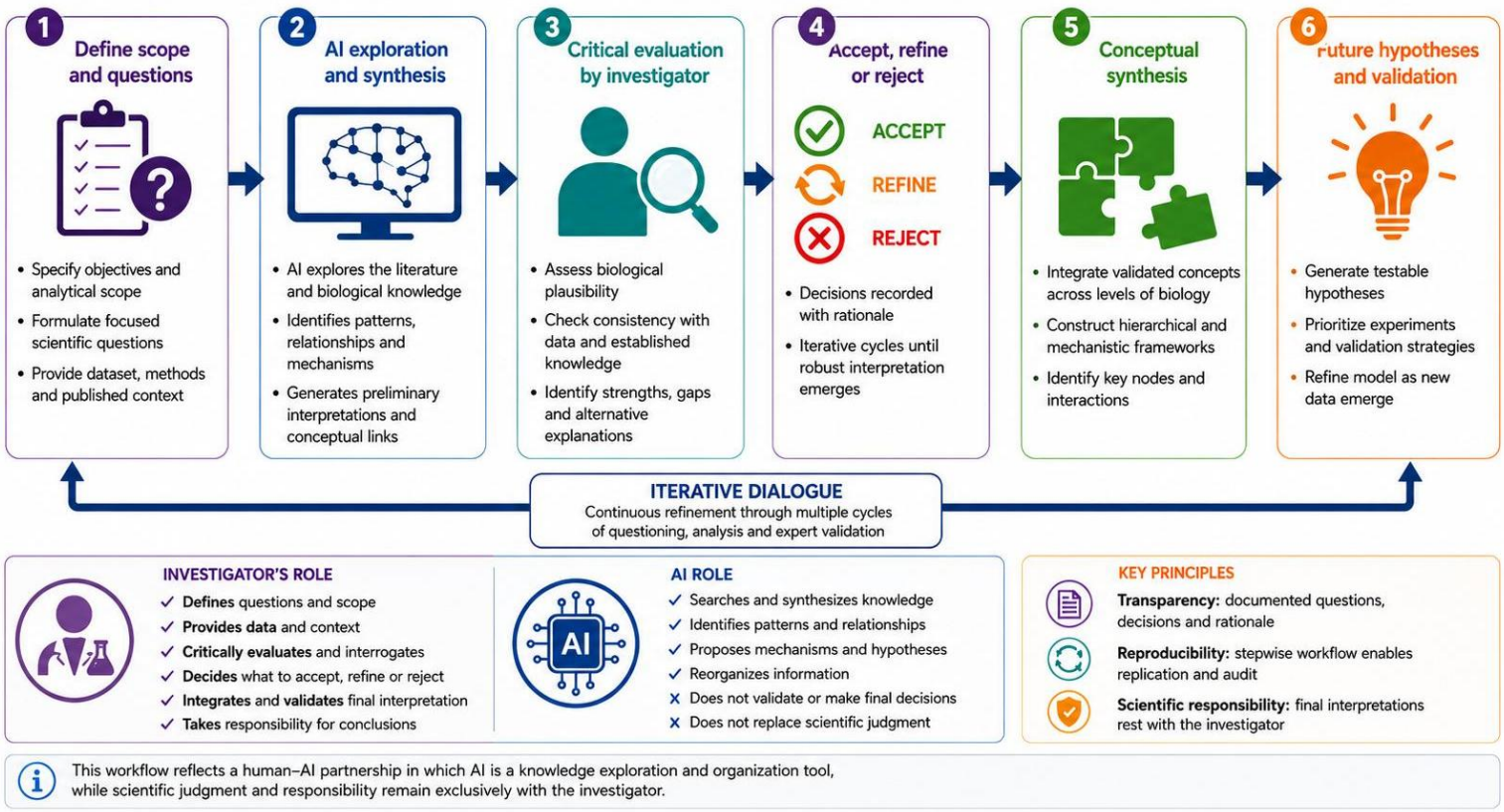


Figure 1. Structured workflow for investigator-led AI-assisted reinterpretation of experimental datasets. The workflow illustrates the analytical strategy adopted in this study. Investigators defined the biological questions, selected the published experimental data, critically evaluated successive AI-assisted outputs, and retained only interpretations consistent with the validated observations and established biological knowledge. The AI platform functioned exclusively as an analytical partner by reorganizing experimentally validated findings, exploring complementary conceptual relationships, and generating hypotheses for future investigation. The final conceptual synthesis resulted from iterative dialogue and continuous investigator supervision rather than autonomous AI inference.

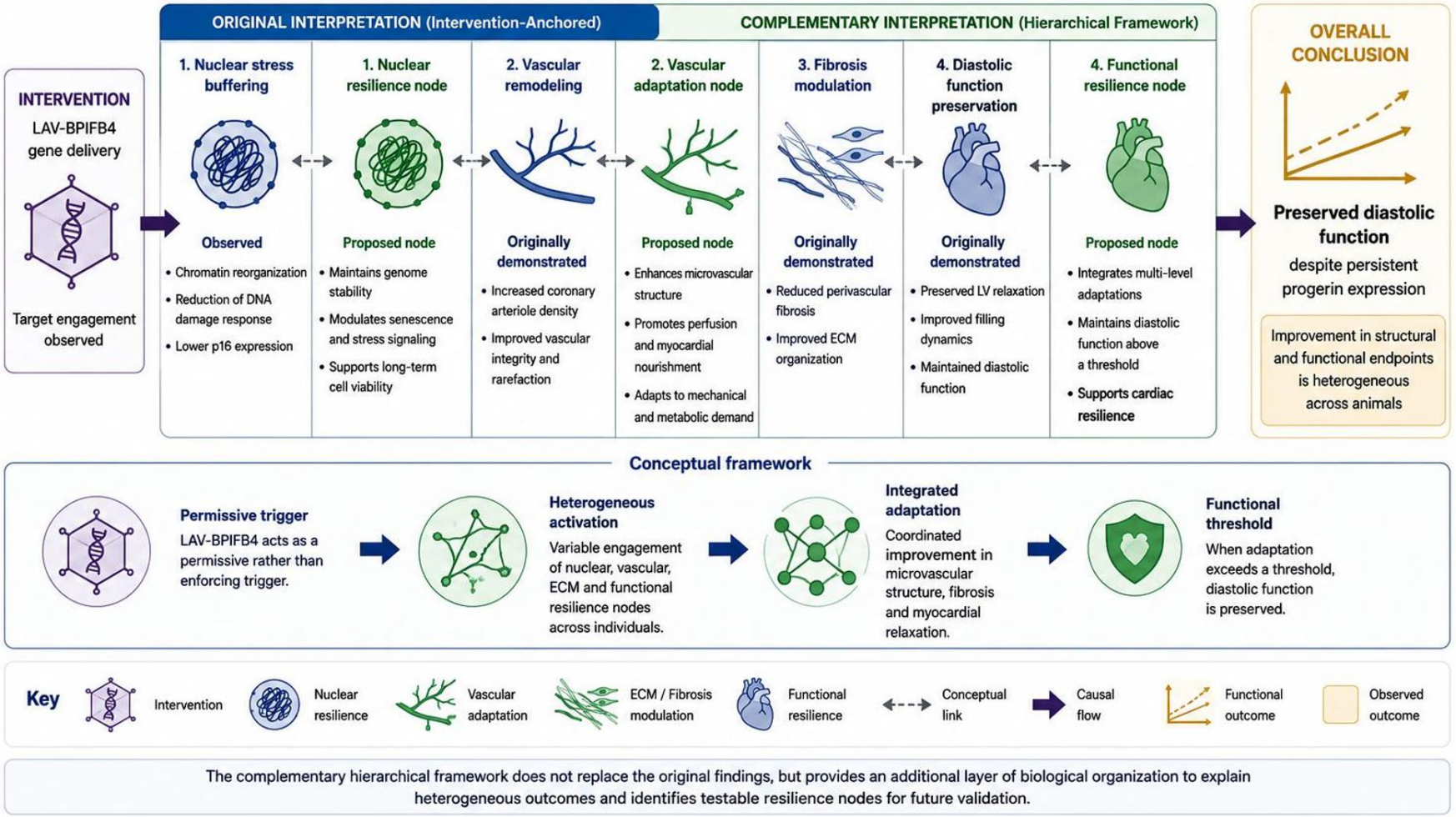


Figure 2. Comparison between the original intervention-anchored interpretation and the complementary hierarchical framework emerging from structured investigator-led analytical dialogue. The central pink pathway represents the original interpretation of the published study, in which LAV-BPIFB4 delivery initiates a sequence of experimentally demonstrated biological events culminating in preservation of left ventricular diastolic function. The blue pathway summarizes the original mechanistic interpretation emphasizing attenuation of nuclear stress and cellular senescence, whereas the green pathway illustrates the complementary hierarchical organization emerging from the structured analytical dialogue, in which vascular remodeling and extracellular matrix adaptation are repositioned as candidate upstream determinants of functional improvement. Solid boxes and arrows indicate experimentally validated observations or established biological relationships. Dashed arrows denote complementary analytical relationships proposed during the dialogue and presented as hypotheses requiring future

experimental validation. The figure illustrates how identical experimental observations can be reorganized into alternative, non-mutually exclusive conceptual frameworks without modifying the original experimental findings.

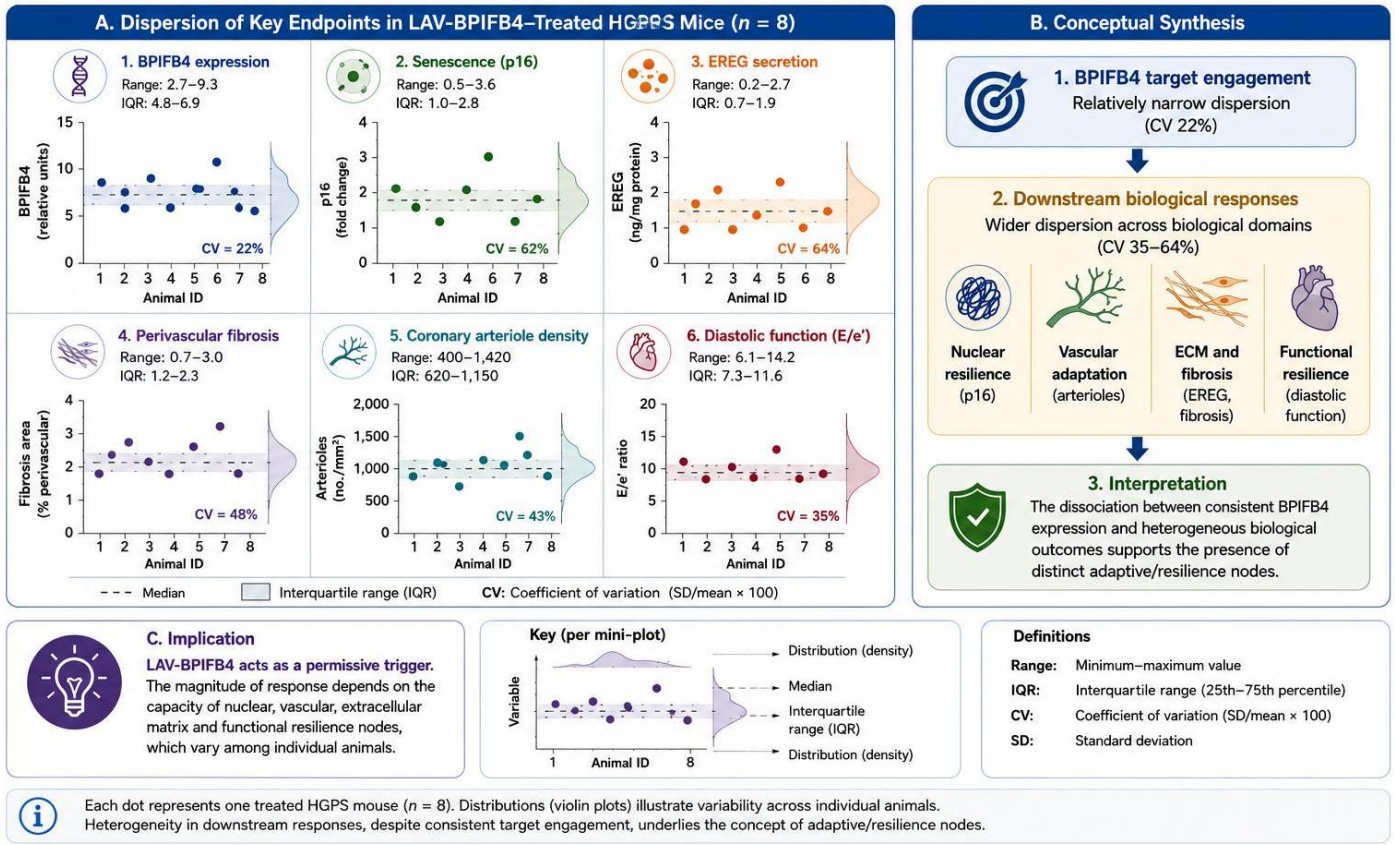


Figure 3. Variability-informed nuclear–vascular resilience framework emerging from structured analytical reinterpretation. Dispersion analysis showed relatively consistent cardiac BPIFB4 target engagement following gene delivery despite substantially greater variability in structural and functional responses. This dissociation suggested that LAV-BPIFB4 may function primarily as a permissive trigger enabling coordinated biological adaptation rather than as a quantitative determinant of therapeutic response. Integration of variability patterns identified four candidate resilience nodes—nuclear stress–senescence adaptation, vascular remodeling, extracellular matrix stiffness, and diastolic performance threshold—that may influence the magnitude of functional improvement. Together, these observations support a variability-informed conceptual framework in which preservation of cardiac function emerges from coordinated interactions among multiple adaptive mechanisms rather than from a simple linear relationship between transgene expression and biological outcome. The proposed resilience nodes represent hypothesis-generating conceptual interpretations that require prospective experimental validation.