Cardiac Tissue–Specific Cell-Free DNA Methylation Profiling for Preclinical Detection of Hereditary ATTR Cardiomyopathy (hATTR-CM): Scientific Rationale and Proposed Phase 1 Pilot Study Design

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Running title: cfDNA Methylation for Preclinical hATTR-CM Detection

Abstract

Background: Hereditary transthyretin amyloid cardiomyopathy (hATTR-CM) is a progressive, infiltrative disease for which three disease-modifying therapies are now approved — tafamidis,[14] acoramidis,[15] and vutrisiran[16] — but whose efficacy is greatest when initiated before significant cardiomyocyte loss. An estimated 10–15% of patients with heart failure with preserved ejection fraction (HFpEF) have undiagnosed ATTR-CM.[1] A large-scale Medicare cohort study found a median diagnostic delay of 494 days from first heart failure diagnosis to ATTR-CM diagnosis — a figure that has not improved from 2016 to 2022 despite expanding awareness and available therapies.[3] The American Heart Association has explicitly identified monitoring in asymptomatic TTR mutation carriers as an area of significant uncertainty, with no accepted definition of progression or response to therapy currently established.[5]

Scientific Rationale: When cardiomyocytes die from amyloid infiltration, they shed cell-free DNA (cfDNA) fragments carrying cardiac-specific methylation signatures into the bloodstream — even before imaging changes appear. Lehmann-Werman et al. established that cardiomyocyte-specific cfDNA methylation patterns are detectable in human plasma during cardiac cell death, with markers validated across all cardiac chambers.[10] We propose applying the CelFiE tissue-of-origin deconvolution algorithm[9] against the Loyfer et al. 39-tissue methylation reference atlas (Nature, 2023)[8] to quantify the cardiac-fraction cfDNA in peripheral blood plasma. Critically, this methodology uses methylation-based tissue-of-origin deconvolution rather than SNP-based approaches, making it applicable in hATTR-CM where cardiac and blood cell cfDNA are genetically identical.

Proposed Study: A Phase 1 prospective observational pilot (N = 125) enrolling five cohorts across 24 months, with plasma collected at months 0, 6, 12, 18, and 24 using standardized pre-analytical protocols. Four specific aims address: reference distribution establishment (Aim 1); subclinical injury detection in asymptomatic hATTR carriers versus matched controls (Aim 2); landmark progression analysis linking cfDNA trajectory to composite cardiac endpoint (Aim 3); and treatment response monitoring on TTR stabilizer therapy as an exploratory pharmacodynamic biomarker (Aim 4). Val122Ile TTR variant carriers are a priority sub-population given their prevalence of approximately 3–4% in African Americans[6] and profound underrepresentation in ATTR research.

Novelty: A systematic search across PubMed, ClinicalTrials.gov, Google Patents, and major cardiovascular conference abstract archives (2018–2026) identified no published studies, registered trials, or patents combining cardiac tissue-specific cfDNA methylation with

asymptomatic hATTR mutation carrier surveillance. ACT-EARLY (NCT06563895), the first Phase 3 ATTR prevention trial enrolling asymptomatic carriers, uses radionuclide imaging as its primary endpoint with no molecular cardiomyocyte injury biomarker component[17] — confirming the gap this proposal directly addresses. The field has explicitly called for longitudinal studies integrating cfNA methylation signatures to distinguish myocardial injury from systemic tissue damage.[24]

Keywords: transthyretin amyloidosis; hereditary ATTR cardiomyopathy; Val122Ile; cell-free DNA; cfDNA methylation; liquid biopsy; tissue-of-origin deconvolution; preclinical biomarker; CelFiE; Loyfer atlas; pharmacodynamic biomarker; health equity; amyloid cardiomyopathy

Introduction

Transthyretin amyloid cardiomyopathy (ATTR-CM) is a progressive, infiltrative disease caused by deposition of misfolded transthyretin (TTR) protein in the myocardium. Two biologically distinct forms exist: hereditary ATTR (hATTR-CM), caused by pathogenic mutations in the TTR gene — with over 100 known variants including Val122Ile, Val30Met, and Thr60Ala — and wild-type ATTR (wtATTR-CM), arising from spontaneous TTR misfolding with aging. An estimated 10–15% of patients with heart failure with preserved ejection fraction (HFpEF) have undiagnosed ATTR-CM.[1,2]

The diagnostic problem is quantified. A large-scale Medicare cohort study of 7,770 beneficiaries found a median delay of 494 days from first heart failure diagnosis to ATTR-CM diagnosis — and this figure did not improve between 2016 and 2022, despite expanding clinical awareness and the introduction of disease-modifying therapies.[3] This stagnation has a direct clinical consequence. Three disease-modifying therapies are now approved: tafamidis,[14] acoramidis,[15] and vutrisiran.[16] All three reduce cardiovascular mortality and hospitalization. But their efficacy is time-dependent: none can reverse amyloid already deposited in the myocardium. By the time a patient meets imaging criteria for hATTR-CM — based on wall thickness, global longitudinal strain, or scintigraphic uptake — significant cardiomyocyte loss and fibrotic remodeling have already occurred, and that damage is irreversible.

Genetic testing can identify hATTR mutation carriers years or decades before symptom onset. The American Heart Association has explicitly identified monitoring in asymptomatic TTR carriers as an area of significant uncertainty, and has stated that no accepted definition of progression or response to therapy in ATTR-CM currently exists.[5] Current biomarker approaches — NT-proBNP, troponin, circulating microRNA[12] — reflect downstream consequences of established disease rather than active cellular injury. No validated blood-based tool exists that can identify which asymptomatic carriers are actively developing myocardial injury. The question is not whether we can treat hATTR-CM. It is whether we can intercept it.

The Val122Ile TTR variant warrants specific attention. Carried by approximately 3–4% of African Americans,[6] it is the most prevalent pathogenic TTR variant in the United States, yet profoundly underrepresented in ATTR research. A tool validated in a cohort enriched for Val122Ile carriers addresses both a scientific gap and a health equity imperative that the field has explicitly recognized.

The field has signaled urgency. ACT-EARLY (NCT06563895) is the first Phase 3 randomized trial evaluating prophylactic acoramidis in approximately 600 asymptomatic hATTR mutation carriers — with first participant dosed in May 2025 and a primary endpoint built around cardiac radionuclide imaging.[17] No molecular cardiomyocyte injury biomarker is included in its protocol. A 2025 review in *Cells* called explicitly for longitudinal studies integrating cfNA methylation

signatures to distinguish myocardial injury from systemic tissue damage in cardiovascular disease.[24] This paper presents the scientific rationale and proposed study design to fill that gap.

Scientific Rationale

The Biology: cfDNA as a Cardiac Injury Signal

Cell-free DNA is released into the bloodstream during cell apoptosis, necrosis, and active remodeling. Every cell type maintains a unique DNA methylation pattern — CpG sites that are hypomethylated in that tissue and hypermethylated in virtually all others. These patterns are established during development, are stable throughout life, and are preserved on cfDNA fragments after cell death, allowing the tissue of origin of circulating cfDNA to be identified from a blood draw.

The foundational methylation atlas by Moss et al.[7] established that cfDNA tissue-of-origin is reliably identifiable using cell-type specific methylation signatures across the human cell repertoire. Lehmann-Werman et al. subsequently demonstrated directly in human plasma that cardiomyocyte-specific methylation patterns serve as a biomarker of cardiac cell death in infarcted and septic patients.[10] The cardiac-specific markers identified — including the FAM101A locus — are consistently demethylated across left ventricle, right ventricle, and right atrium, confirming pan-cardiomyocyte specificity regardless of cardiac chamber. Liu et al. further confirmed cardiac-specific cfDNA detection in 54 of 116 acute MI patients at hospital admission using independent heart-specific hypermethylation loci.[11]

An important signal-magnitude caveat: the cardiac cfDNA elevation in subclinical hATTR-related cardiomyocyte injury is expected to be substantially lower than in acute MI, where massive acute necrosis generates a large signal. Amyloid infiltration drives predominantly apoptotic cardiomyocyte death — slow, progressive, and continuous rather than acute. Aim 1 of this study is specifically designed to establish the hATTR disease-specific reference distribution before applying the assay to asymptomatic carriers. If the assay cannot detect a signal in established symptomatic disease, it will not be applied to the surveillance population.

cfDNA half-life in plasma ranges from 15 to 120 minutes in published studies,[22] mediated by plasma nucleases, hepatic Kupffer cell uptake, and renal filtration. Renal clearance is size-dependent: impaired GFR prolongs cfDNA circulating half-life and artifactually elevates plasma levels across all tissue fractions. This mechanism is the biological basis for the eGFR exclusion criterion and pre-analytical standardization protocol embedded in this study's design.

The Method: CelFiE Algorithm and Loyfer Atlas

Tissue-of-origin deconvolution will be performed using the CelFiE algorithm,[9] applied against the Loyfer et al. 39-tissue methylation reference atlas (Nature, 2023).[8] The atlas includes cardiac muscle as a validated discrete tissue type using cardiomyocyte samples across six biological replicates, with cardiac-specific markers identified at the MYL4 locus and others showing consistent hypomethylation in cardiomyocytes and hypermethylation in all other profiled tissues — including blood, liver, skeletal muscle, lung, and kidney.

A cfDNA fragment is classified as cardiac-origin if it carries concordant hypomethylation at ≥3 cardiac-specific CpG loci within a single sequencing read. The cardiac cfDNA fraction is expressed as the percentage of total plasma cfDNA fragments meeting this threshold per sample.

This approach is methodologically distinct from SNP-based cfDNA analysis — exemplified by CareDx AlloSure in cardiac transplant rejection monitoring — which requires genetic distinctiveness between source tissue and background cfDNA. In hATTR-CM, cardiac cfDNA and circulating blood cell cfDNA are genetically identical. Methylation-based tissue-of-origin deconvolution is the correct methodological class for this scientific question. The cardiac transplant cfDNA literature is cited in this proposal solely as biological precedent for signal magnitude — confirming that dying cardiomyocytes produce a quantifiable plasma cfDNA signal[18] — not as methodological precedent.

The Gap: What Existing Approaches Cannot Do

Table 1 summarizes the current biomarker landscape and the proposed advance. First-generation protein biomarkers (NT-proBNP, troponin) are nonspecific and detectable only after significant myocardial strain. Second-generation circulating miRNA and extracellular vesicle studies demonstrated field interest but were limited to established symptomatic disease with no tissue-of-origin specificity and no preclinical application. The proposed third-generation approach — cardiac tissue-specific cfDNA methylation deconvolution — is the first methodology designed for application in asymptomatic genetically at-risk carriers.

Table 1. Biomarker approaches in hATTR-CM and the proposed third-generation advance.

Biomarker Class	Limitation in hATTR	Generation
Protein biomarkers (NT-proBNP, troponin)	Nonspecific; elevated only after significant myocardial strain; no tissue-of-origin identification	1st Generation
Circulating miRNA (Derda et al., 2018)	Established disease only; small cohorts; no tissue-of-origin specificity; no preclinical data	2nd Generation
Extracellular vesicles (NCT07314268, Brazil, 2024)	EV proteomics in symptomatic patients; excludes DNA analysis; cross-sectional only	2nd Generation
Cardiac imaging (echo, CMR, scintigraphy)	Requires structural changes already present; cannot detect subclinical injury; radiation with scintigraphy	Structural (late)
Cardiac cfDNA methylation (CelFiE + Loyfer Atlas)	**Tissue-specific; detects active cardiomyocyte injury before structural changes; applicable in asymptomatic genetically at-risk carriers**	**3rd Generation — PROPOSED**

Specific Aims

The four aims are sequentially dependent: Aim 1 validates the assay; Aim 2 applies it to the target population; Aim 3 tests its prognostic utility; Aim 4 explores its pharmacodynamic application. This structure ensures that each subsequent aim is built on validated preceding steps rather than assumed to work in a novel disease context.

Aim 1 — Establish the cfDNA Methylation Reference Distribution

Characterize cardiac-specific methylation profiles in patients with confirmed symptomatic ATTR-CM (both hATTR and wtATTR, no stabilizer therapy) versus healthy controls, establishing a disease-context reference distribution and empirical 95th percentile threshold for cardiac tissue-derived cfDNA.

Why Aim 1 must come first: This is the analytical validation step. If the CelFiE algorithm and Loyfer atlas cannot detect a cardiac cfDNA signal in established symptomatic amyloid disease, the assay has no scientific basis for application to asymptomatic carriers in Aim 2. The threshold for "elevated" in all subsequent aims is derived empirically here — not assumed from other disease contexts.

Aim 2 — Detect Subclinical Myocardial Injury in Asymptomatic hATTR Carriers

Quantify cardiac-fraction cfDNA methylation in asymptomatic hATTR mutation carriers relative to age-, sex-, and comorbidity-matched genotype-negative controls, adjusting for renal function, cardiovascular risk burden, and standardized pre-collection conditions, to determine whether a statistically distinguishable cardiac cfDNA signal exists prior to structural disease onset. Correlate signal magnitude with early imaging abnormalities including global longitudinal strain (GLS) and extracellular volume fraction (ECV) on CMR.

Why this comparison is valid: Specificity does not come from the molecular signal alone — it comes from population design. In a genetically confirmed hATTR carrier who is imaging-negative and at low cardiovascular risk, a cardiac cfDNA excess versus matched genotype-negative controls, after covariate adjustment, is most parsimoniously explained by TTR mutation status. The genotype-negative controls are matched within ±5 years for age, sex, and comorbidity burden to isolate the genetic contribution.

Aim 3 — Landmark Progression Analysis

Using a pre-specified landmark analysis, determine whether a rising cardiac cfDNA trajectory between baseline and 12 months is independently associated with a composite cardiac progression event occurring between 12 and 24 months in asymptomatic hATTR carriers, after covariate adjustment.

Composite progression endpoint: New GLS deterioration below –18%; new late gadolinium enhancement (LGE) or extracellular volume fraction (ECV) above 30% on CMR; septal thickness increase ≥2mm; or sustained NT-proBNP doubling. Events adjudicated by an imaging cardiologist blinded to all cfDNA results.

Why landmark design: Equal 12-month windows on each side of the landmark prevents reverse causation — the cfDNA exposure window (0→12 months) is completely defined before the progression outcome window (12–24 months) begins. This establishes temporal precedence, the appropriate evidentiary standard for a Phase 1 biomarker progression study. Given pilot sample size, findings are designated hypothesis-generating and intended to power a subsequent validation cohort.

Aim 4 — Treatment Response Monitoring (Exploratory)

Determine whether cardiac cfDNA fraction responds dynamically to TTR stabilizer therapy (tafamidis, acoramidis) in symptomatic hATTR-CM patients over 24 months.

The clinical gap this aim addresses: Current pharmacodynamic monitoring of patients on TTR stabilizers relies on serum TTR (protein stabilization), NT-proBNP and troponin (cardiac stress), and echocardiography (structural change).[19] None measure whether cardiomyocytes are actively dying at the time of the blood draw. In patients treated with TTR silencers, serum TTR becomes decoupled from cardiac risk and cannot confirm cellular cardiac protection.[20] A declining cardiac cfDNA fraction on therapy would be the first direct evidence of cellular-level cardiac protection. A rising trajectory despite therapy would be the first molecular signal of treatment failure before imaging changes appear. Aim 4 findings are designated hypothesis-generating.

Proposed Methods

Study Design

Phase 1 prospective observational pilot study with longitudinal follow-up (24 months). Primary objective: signal detection and effect size estimation in hATTR carriers. The primary deliverable is the empirically measured cardiac cfDNA effect size in this population, which will power a subsequent adequately-sized validation cohort. IRB approval and ClinicalTrials.gov registration will be obtained prior to enrollment.

Study Population

One hundred and twenty-five adult participants across five pre-defined cohorts (Table 2). Val122Ile carriers are a priority sub-population within the asymptomatic hATTR carrier cohort. Baylor Scott & White Health's patient population in the Dallas-Fort Worth metroplex provides geographic access to enroll a cohort that addresses this health equity gap directly.

Table 2. Study cohort structure.

Cohort	N	Scientific Purpose	Analysis Role
Symptomatic hATTR/wtATTR-CM (no stabilizer)	25	Aim 1: Reference cfDNA distribution in established amyloid disease	Reference distribution; empirical 95th %ile threshold
Asymptomatic hATTR carriers — stabilizer-naïve (Val122Ile priority)	25	Aims 2 & 3: Detect subclinical injury; landmark progression analysis	**PRIMARY: pre-specified confirmatory analyses**
hATTR-CM on tafamidis or acoramidis	25	Aim 4: Pharmacodynamic treatment monitoring	Exploratory; hypothesis-generating
Genotype-negative controls (±5 yr matched)	25	Aims 1 & 2: Age/sex/comorbidity-matched comparator	Comparator arm for Aim 2 primary analysis

Healthy controls	25	Aim 1: Population-level cfDNA lower bound	Lower bound reference range

Assessment Schedule and Pre-Analytical Protocol

Peripheral blood (plasma, 10 mL) will be collected at five pre-specified timepoints: baseline (month 0), month 6, month 12 (primary landmark timepoint), month 18, and month 24. Full clinical assessment and cardiac imaging (echocardiography; cardiac MRI where indicated) will be performed at months 0, 12, and 24. Months 6 and 18 involve cfDNA collection and laboratory panels only, consistent with the hypothesis that cfDNA rises before structural imaging changes become apparent.

Pre-analytical protocol: Blood will be collected in Streck Cell-Free DNA BCT tubes (preferred; stabilizes cfDNA for up to 14 days, preventing white blood cell lysis) or EDTA tubes with plasma separation within 2 hours of collection. A double-centrifugation protocol will be applied: 1,600g for 10 minutes followed by 16,000g for 10 minutes. Plasma aliquots stored at –80°C until batch analysis. Time from blood draw to first centrifugation will be recorded at every timepoint as a covariate. Pre-collection instructions: no vigorous exercise 48 hours prior to each draw; morning collection following an overnight or light fast.

A trigger-based unscheduled clinical assessment will be initiated within 30 days for any participant demonstrating a cfDNA cardiac fraction rise of ≥50% from individual baseline, to protect participants and capture early progressors who may not wait for the next scheduled visit.

Primary Outcome Measure

Cardiac-fraction cfDNA methylation score: the percentage of total plasma cfDNA fragments classified as cardiac-muscle origin using tissue-of-origin deconvolution (CelFiE algorithm)[9] applied against the Loyfer et al. 39-tissue methylation reference atlas (Nature, 2023).[8] Cardiac-origin classification requires concordant hypomethylation at ≥3 cardiac-specific CpG loci within a single sequencing read. Analytical validation confirmed in Aim 1 before application in Aims 2–4.

Confound Mitigation

Six strategies are embedded by design to address non-amyloid sources of cardiac cfDNA signal:

- Age: genotype-negative controls matched within ±5 years; age included as continuous covariate in all models.
- Renal function: eGFR at every timepoint; exclude eGFR <45 mL/min/1.73m² at enrollment; mechanistic basis is renal-mediated alteration of cfDNA clearance kinetics.[22]
- Subclinical CAD and hypertension: ASCVD 10-year risk score and coronary artery calcium as covariates; sensitivity analysis in low-risk subgroup (ASCVD <10%, no hypertensive heart disease).
- Exercise-induced cfDNA spikes: 48-hour pre-draw restriction; morning collection; time-of-day recorded as covariate.

- Pre-analytical standardization: Streck tubes or 2-hour EDTA processing window; double-centrifugation; processing interval recorded as covariate. This eliminates between-participant pre-analytical variability as a systematic confounder.
- Threshold definition: the cutoff for 'elevated' cardiac cfDNA is derived empirically from Aim 1 data, not imposed a priori. This eliminates circular reasoning in the Aim 2 analysis.

Power Calculation

Precise power calculation at the concept stage is not possible because the cardiac cfDNA effect size in asymptomatic hATTR carriers is unknown — which is the scientific rationale for this pilot. Biological precedent from cardiac transplant cfDNA monitoring (SNP-based, cited for signal magnitude only) reports a median donor-derived fraction of 0.07% without rejection and 0.17% with acute rejection.[18] If subclinical hATTR-related injury produces a 2–3 fold elevation above the matched-control baseline, that represents Cohen's $d > 0.8$. At $n = 25$ per group, the study has approximately 80% power to detect $d = 0.8$ at $\alpha = 0.05$, two-sided. The primary deliverable of this pilot is the empirically measured effect size in hATTR carriers to power the subsequent validation cohort.

Sequencing Platform

A tiered cost-efficient strategy: Aim 1 ($n = 50$, atlas construction): whole-genome bisulfite sequencing (WGBS; ~$500–1,500/sample; Zymo-Seq Trio Kit). Aims 2–4 (575 samples): targeted bisulfite sequencing of the top 100–200 cardiac-specific CpG sites identified in Aim 1 (~$150–$250/sample; Zymo Research or Daicel Arbor Biosciences myBaits Custom Methyl-Seq). Enzymatic methylation sequencing (EM-seq; NEB) is the recommended scale-up option at approximately $80–$200/sample.[23] Total estimated laboratory budget: $224K–$299K for a 24-month study.

Discussion

This paper presents the scientific rationale and proposed study design for a first-in-disease application of tissue-specific cfDNA methylation profiling to hereditary ATTR cardiomyopathy. The central premise — that amyloid-driven cardiomyocyte injury produces a detectable, tissue-specific circulating molecular signal before structural changes appear on cardiac imaging — rests on three converging lines of evidence: the biology of cfDNA methylation tissue-of-origin identification established by Moss et al. and Lehmann-Werman et al.,[□,1□] the validated CelFiE deconvolution algorithm,[□] and the Loyfer 39-tissue methylation reference atlas.[□]

The specificity argument deserves explicit address. The cardiac cfDNA methylation signal is not disease-specific at the molecular level — a cardiomyocyte dying from amyloid infiltration releases methylation-identical cfDNA to one dying from ischemia. Specificity in this study is achieved through population design: a rising cardiac cfDNA fraction in a genetically confirmed hATTR carrier who is imaging-negative and at low cardiovascular risk, after covariate adjustment for age, renal function, and ASCVD burden, is most parsimoniously explained by their TTR mutation status. This is the same logical framework used in every population-based biomarker study in medicine. The five confound mitigation strategies embedded in the design —

and the sixth of defining “elevated” empirically from Aim 1 data — address the most common reviewer objections to this class of study.

The Val122Ile finding deserves specific discussion. The variant is carried by approximately 3–4% of African Americans[6] — representing millions of individuals at elevated genetic risk for hATTR-CM in the United States. This population is systematically underrepresented in ATTR clinical trials and biomarker studies. A surveillance tool validated in a cohort enriched for Val122Ile carriers at a demographically appropriate institution does not merely fill a scientific gap. It addresses a health equity failure that the field has named but not yet corrected.

Aim 4 addresses the treatment monitoring gap. No standardized molecular protocol exists to confirm that TTR stabilizer therapy is protecting the heart at the cellular level in an individual patient.[19] Current monitoring measures protein stabilization (serum TTR), cardiac stress (NT-proBNP, troponin), or structural change (echo, CMR) — none of which reflect whether cardiomyocytes are actively dying at the time of the draw. In patients treated with TTR silencers, serum TTR itself becomes decoupled from cardiac risk[20] — and CMR-based monitoring, while emerging, requires specialized imaging not available for frequent longitudinal use.[21] A declining cardiac cfDNA fraction on tafamidis or acoramidis would be the first direct evidence that the drug is protecting the heart at the cardiomyocyte level. A rising trajectory despite therapy would be the first molecular signal of treatment failure before imaging changes appear. This positions cardiac cfDNA methylation not just as a surveillance tool but as a pharmacodynamic biomarker with direct commercial value to all three approved therapy sponsors.

ACT-EARLY[17] represents both a validation of this proposal’s scientific premise and an immediate collaborative opportunity. Six hundred asymptomatic hATTR carriers are being randomized with longitudinal plasma collection and no cfDNA component in the protocol. A cfDNA methylation sub-study added to ACT-EARLY would address Aims 2 and 3 with an already-enrolled, well-characterized population, dramatically compressing the timeline from concept to data. Dr. Justin Grodin at UT Southwestern Medical Center (Dallas) is independently running two NHLBI-funded complementary studies: NCT05489549 (subclinical hATTR-CM in Val122Ile carriers, with Cleveland Clinic and Columbia University) and NCT07196839 (TAD1 circulating TTR aggregate monitoring on stabilizer therapy). TAD1 measures whether TTR protein is aggregating; cardiac cfDNA methylation measures whether cardiomyocytes are dying as a result. These approaches are biologically complementary and methodologically non-overlapping — a natural collaborative pairing.

Future Directions

A longer-term scientific vision emerges from the biology of this proposal. Single-timepoint cfDNA measurement reflects cardiomyocyte injury over the preceding 15–120 minutes. The longitudinal trajectory design in this study partially addresses that limitation by measuring the slope of change rather than a single value. But a more powerful concept is possible: a time-integrated cardiac injury index that behaves, in principle, like HbA1c for the heart.

The analogy is conceptual rather than mechanistic — HbA1c works through irreversible biochemical accumulation of glycated hemoglobin over the 90–120 day red cell lifespan, while cfDNA does not accumulate. But the clinical vision is the same: a signal that reflects cumulative myocardial injury over weeks to months rather than the preceding hour. Two emerging approaches make this scientifically tractable. First, cfDNA fragmentomics: apoptotic cell death — the dominant mechanism in amyloid infiltration — produces characteristically sized fragments at approximately 147–200 base pairs through caspase-activated cleavage at internucleosomal sites, while necrotic or acute stress-induced death produces larger, irregular fragments. A fragmentomics filter selecting only apoptotic-pattern cardiac cfDNA would preferentially enrich the chronic amyloid-driven signal and attenuate acute-stress noise. Second, nucleosome-level

histone modification analysis: cfDNA is histone-bound in plasma, and circulating nucleosomes maintain histone modifications from their tissue of origin. Recent PNAS work by Lo et al. (2024) demonstrated that histone modifications of circulating nucleosomes can be deduced noninvasively from cfDNA fragmentation patterns and used to infer tissue-specific pathological processes. A cardiac-specific nucleosome histone modification signature, potentially with a longer circulating half-life than naked cfDNA fragments, may provide a more temporally integrated signal than single-fragment methylation analysis alone.

These approaches are not components of the current pilot — the pilot is designed to establish whether a detectable cardiac cfDNA signal exists in asymptomatic hATTR carriers at all. But if the pilot confirms signal presence, the next generation of investigation would develop the temporal integration framework as the precision medicine equivalent of HbA1c for cardiac amyloid surveillance. The field has explicitly called for this work.[2□]

Limitations

This study has three principal limitations that are acknowledged as the scientific rationale for Phase 1 design rather than flaws in the approach. First, the pilot sample size (n = 25 per group) is powered for signal detection, not validation — a larger adequately-powered validation cohort is the planned second step. Second, no hATTR-specific pilot cfDNA data exist; the power calculation is anchored to analogous biology from transplant and MI literature, and the true effect size is unknown prior to this study. Third, CelFiE deconvolution requires bioinformatics infrastructure and does not yet exist as a point-of-care assay — implementation will require dedicated computational support and batch processing, which is appropriate for a research protocol but limits immediate clinical translation.

Required Statements

Data Availability Statement

No data were generated or analyzed in the preparation of this manuscript. This is a research concept paper presenting scientific rationale and a proposed study design. Data will be made available upon study completion in accordance with applicable data sharing policies.

Ethics Statement

This manuscript presents a research protocol and scientific concept paper. No human participants were enrolled, studied, or involved in its preparation. No patient data were collected or analyzed. Institutional Review Board approval and ClinicalTrials.gov registration will be obtained prior to participant enrollment, in accordance with the Declaration of Helsinki and applicable federal regulations governing human subjects research in the United States of America. This is a prospective observational study with no intervention to be assigned to participants; and therefore, does not require prospective clinical trial registration prior to IRB submission.

Competing Interests

The author declares no competing interests.

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Author Contributions

Hakeem Arzu, MD: Concept origination, systematic literature search, study design, manuscript preparation. This manuscript was prepared solely by the corresponding author.

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