Journal Club

The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med. 2025;392(1):33-44.

Vutrisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy

Authors:
M. Fontana, J.L. Berk, J.D. Gillmore, R.M. Witteles, M. Grogan, B. Drachman, T. Damy, P. Garcia-Pavia, J. Taubel, S.D. Solomon, F.H. Sheikh, N. Tahara, J. González-Costello, K. Tsujita, C. Morbach, Z. Pozsonyi, M.C. Petrie, D. Delgado, P. Van der Meer, A. Jabbour, A. Bondue, D. Kim, O. Azevedo, S. Hvitfeldt Poulsen, A. Yilmaz, E.A. Jankowska, V. Algalarrondo, A. Slugg, P.P. Garg, K.L. Boyle, E. Yureneva, N. Silliman, L. Yang, J. Chen, S.A. Eraly, J. Vest, and M.S. Maurer, for the HELIOS-B Trial Investigators*

Speaker：陳品潔 藥師

Advisor：李雅涵 藥師

Date：2025/07/17

Outline

1. Background
- Pathological mechanisms of ATTR-CM
- Types of ATTR amyloidosis
- Diagnosis and prognosis of ATTR-CM
- General management for ATTR-CM
- Disease-specific therapy for ATTR-CM

2. Methods
- Trial oversight
- Patient inclusion and exclusion criteria
- Trial design
- End points：Primary, Secondary, Exploratory, Safety
- Statistical analysis

3. Results
- Baseline Characteristics
- Primary end point
- Secondary end points
- Exploratory end points
- Safety

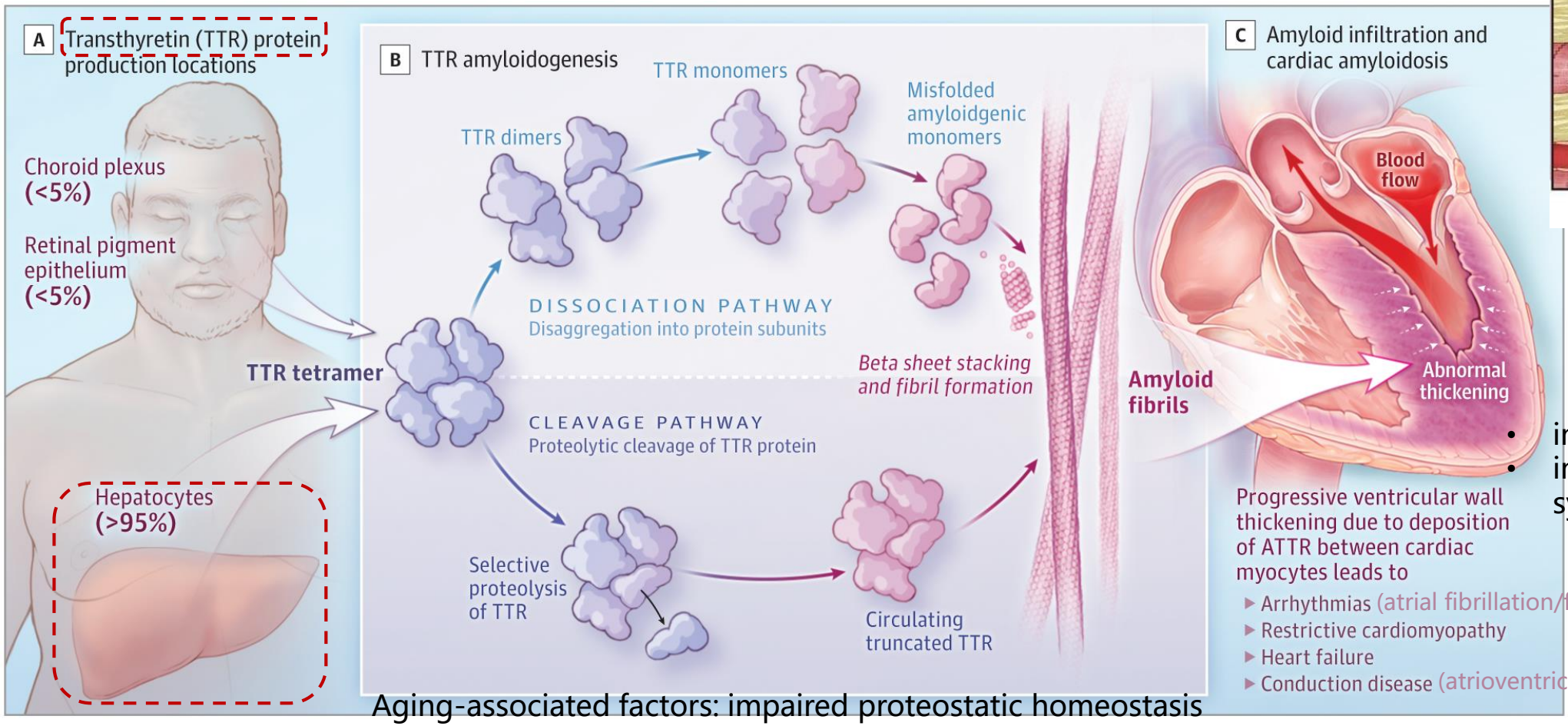
4. Discussion

5. Appraisal
- CASP (+Cochrane RoB2)

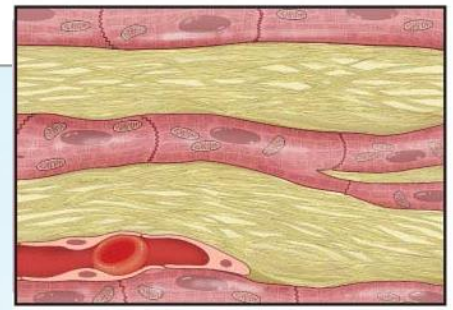
Background

Transthyretin amyloid cardiomyopathy (ATTR-CM)

Transthyretin(Prealbumin): transports thyroid hormone and the retinol (vitamin A)-binding protein 4 complex(RBP4).



Amyloid deposits in myocardium



- impaired diastolic function
- impaired longitudinal systolic function

Aging-associated factors: impaired proteostatic homeostasis (including endoplasmic reticulum-associated degradation of misfolded TTR)

JAMA. 2024;331(9):778-791.

Types of ATTR amyloidosis

Wild-type (wtATTR) amyloidosis (~75%)

Hereditary (hATTR) amyloidosis (~25%)

Table 1. Genetic Variants Associated With ATTR Amyloidosis in the US

Genotype	Estimated frequency	Estimated penetrance	Typical age at onset, y	Clinical characteristic
Wild-type (no pathological genetic variants)	• 12% (95% CI, 6%-20%) in HFpEF; 10% (95% CI, 6%-15%) in HFrEF • 7% (95% CI, 5%-10%) in surgery for carpal tunnel syndrome • 7% (95% CI, 5%-18%) in hypertrophic cardiomyopathy phenotype • 8% (95% CI, 5%-13%) in severe aortic stenosis • 21% (95% CI, 7%-39%) in autopsy series of "unselected" older individuals[17]	Not genetically inherited	>60	Predominantly cardiac
Val122Ile (also known as V122I or pV142I)	3.4% African American individuals[18]	~7% to 100% >age 60 y, increases with age, males > females[19-21]	>65	Predominantly cardiac
Val30Met (also known as V30M or pV50M, early onset, endemic regions)	Most common worldwide, 4% in northern regions of Sweden, 1:500 northern regions of Portugal[18]	>90%	<50	Predominantly neurologic
Val30Met (late-onset, endemic and nonendemic regions)	1:1 000 000 in Japan and Sweden[18]	>60%	>50	Mixed
Thr60Ala (also known as T60A or pT80A)	1% County Donegal, Republic of Ireland[18]	>90%	>50	Mixed

Abbreviations: ATTR, amyloidosis from transthyretin; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction.

*More than 130 gene variants in TTR have been identified.

JAMA. 2024;331(9):778-791.

Primary clinical manifestations of ATTR amyloidosis

Organ system	Clinical manifestation
Cardiovascular	• Atrioventricular block • Congestive heart failure • Arrhythmia (atrial fibrillation/flutter, ventricular tachycardia)
Nervous hATTR>wtATTR	• Peripheral neuropathy (weakness/numbness in hands and feet) • Autonomic neuropathy (orthostatic hypotension, erectile dysfunction, sweating abnormalities)
Gastrointestinal	Dysmotility (diarrhea, constipation, nausea/vomiting, early satiety)
Musculoskeletal	• Spinal stenosis • Carpal tunnel syndrome • Ligamentous rupture
Kidney	Acute and chronic kidney disease (cardiorenal syndrome, hemodynamically mediated kidney impairment)

JAMA. 2024;331(9):778-791.

Diagnosis of ATTR Cardiomyopathy

Clinical scenarios in which cardiac amyloidosis from transthyretin (ATTR) should be considered

unexplained LV hypertrophy

- ▸ Increased left ventricular wall thickness above normal for sex

and

- ▸ Clinical features (with or without HF)
  - → Heart failure in adults aged >60 y
  - Significant aortic stenosis in adults aged >65 y
  - Hypotension or normotension if previously hypertensive
  - → Peripheral or autonomic neuropathy
  - → Bilateral carpal tunnel syndrome
  - → Lumbar spinal stenosis
  - → Biceps tendon rupture
  - Family history of cardiomyopathy
  - Hypertrophic cardiomyopathy in older adults
  - History of multiple orthopedic surgeries (hip, knee, or shoulder)

- Echocardiography
- Cardiovascular magnetic resonance (CMR) imaging

- ▸ Electrocardiogram (ECG) or cardiac imaging findings

ECG
- Discordance between QRS voltage on ECG and wall thickness
- Low voltage — Abnormal conduction
- Pseudoinfarcts on ECG
- Atrioventricular conduction disease

Echo
- Restrictive phenotype; small chambers and enlarged atrium
- Reduced longitudinal strain with apical sparing
- Low tissue doppler velocities
- Increased atrial septal thickness

MRI
- Late gadolinium enhancement — myocardial tissue fibrosis or scarring
- Increased extracellular volume fraction — myocardial infiltration
- Increased native myocardial T1 time

Echocardiography→hypertrophy

- **increased wall thickness ≥ 12 mm**
- **LVEF < 60%**
- **relative wall thickness > 0.57 mm**

→associated with ATTR cardiomyopathy in male patients older than 60 years who do not have a history of hypertension.

JAMA. 2024;331(9):778-791.

Diagnosis of ATTR Cardiomyopathy

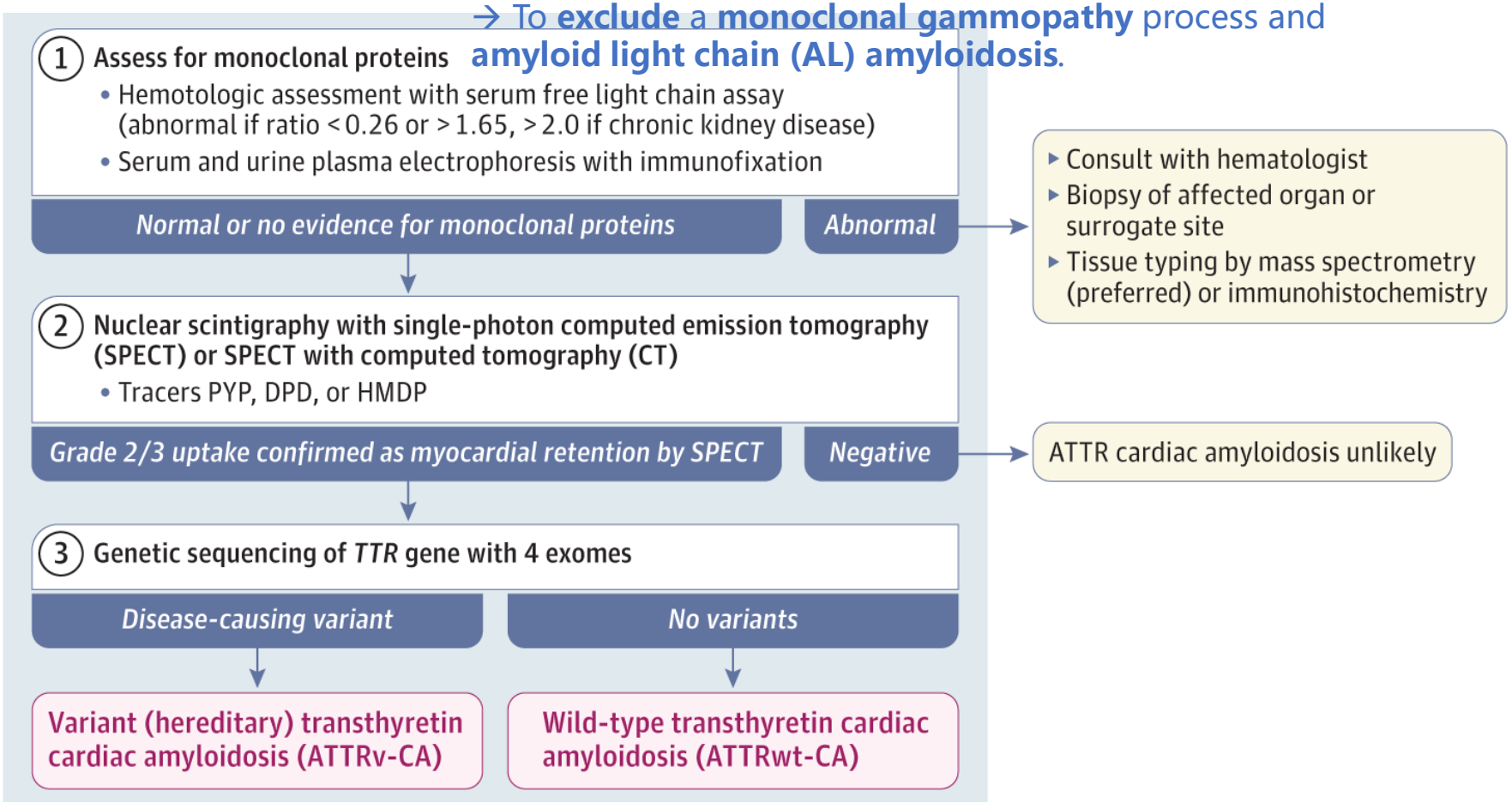
Invasive diagnostic test:
→ **Endomyocardial biopsy**

- Presence of Congo red staining
- Laser capture tandem mass spectrometry demonstrating TTR protein in amyloid deposits

→ Carries risk of myocardial perforation/tamponade, arrhythmia, and vascular access complication.

Noninvasive diagnostic test:

→ To **exclude** a **monoclonal gammopathy** process and **amyloid light chain (AL) amyloidosis**.

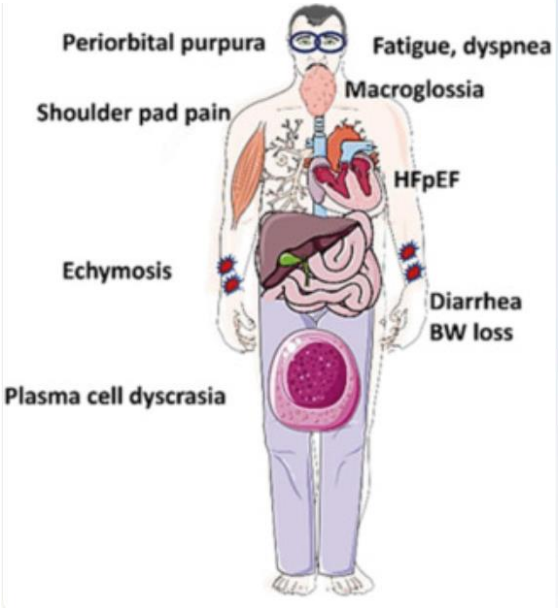


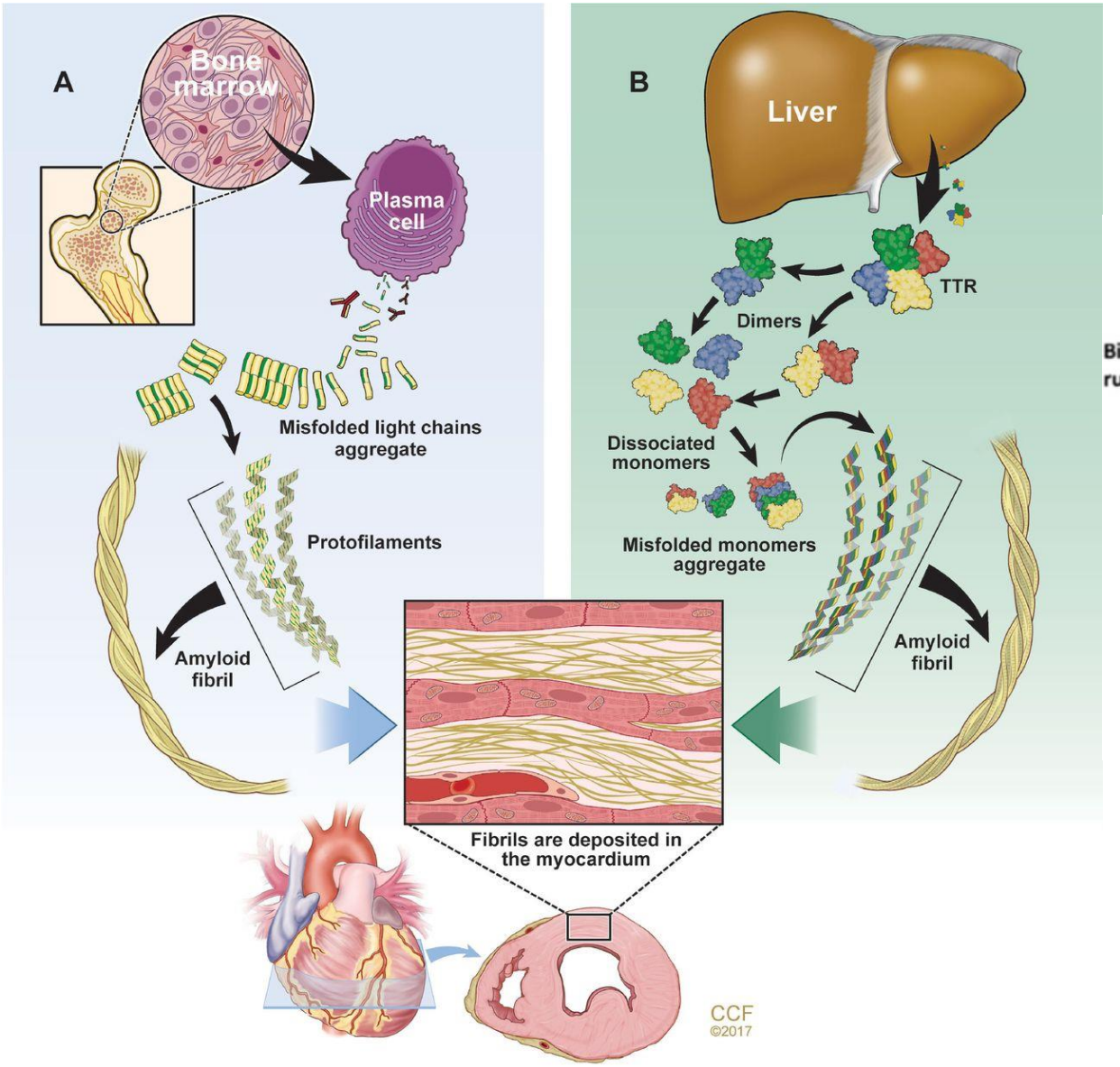
PYP: pyrophosphate
DPD: 3,3-diphosphono-1,2-propanodicarboxylic acid
HMDP: hydroxymethylene diphosphonate

JAMA. 2024;331(9):778-791. *J Nucl Cardiol.* 2021;28(2):604-609.

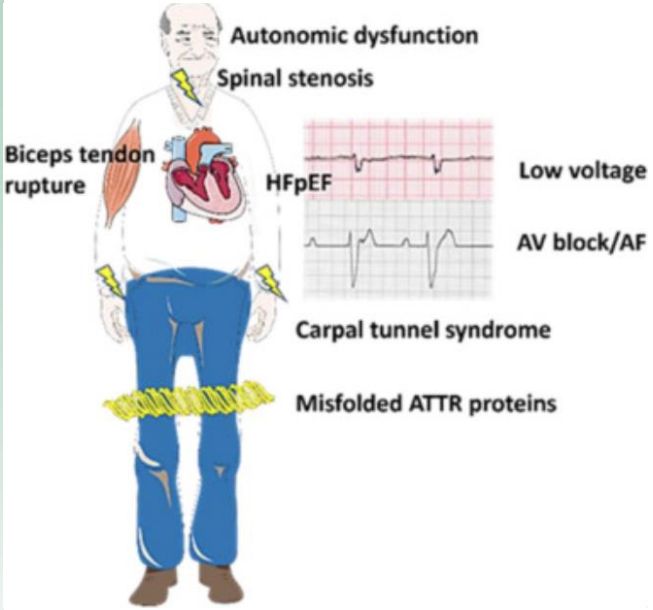
AL amyloidosis vs ATTR amyloidosis

Light-Chain (AL) amyloidosis





ATTR amyloidosis



Cleve Clin J Med. 2017;84(12 Suppl 3):12-26.

Acta Cardiol Sin. 2023;39(4):511-543.

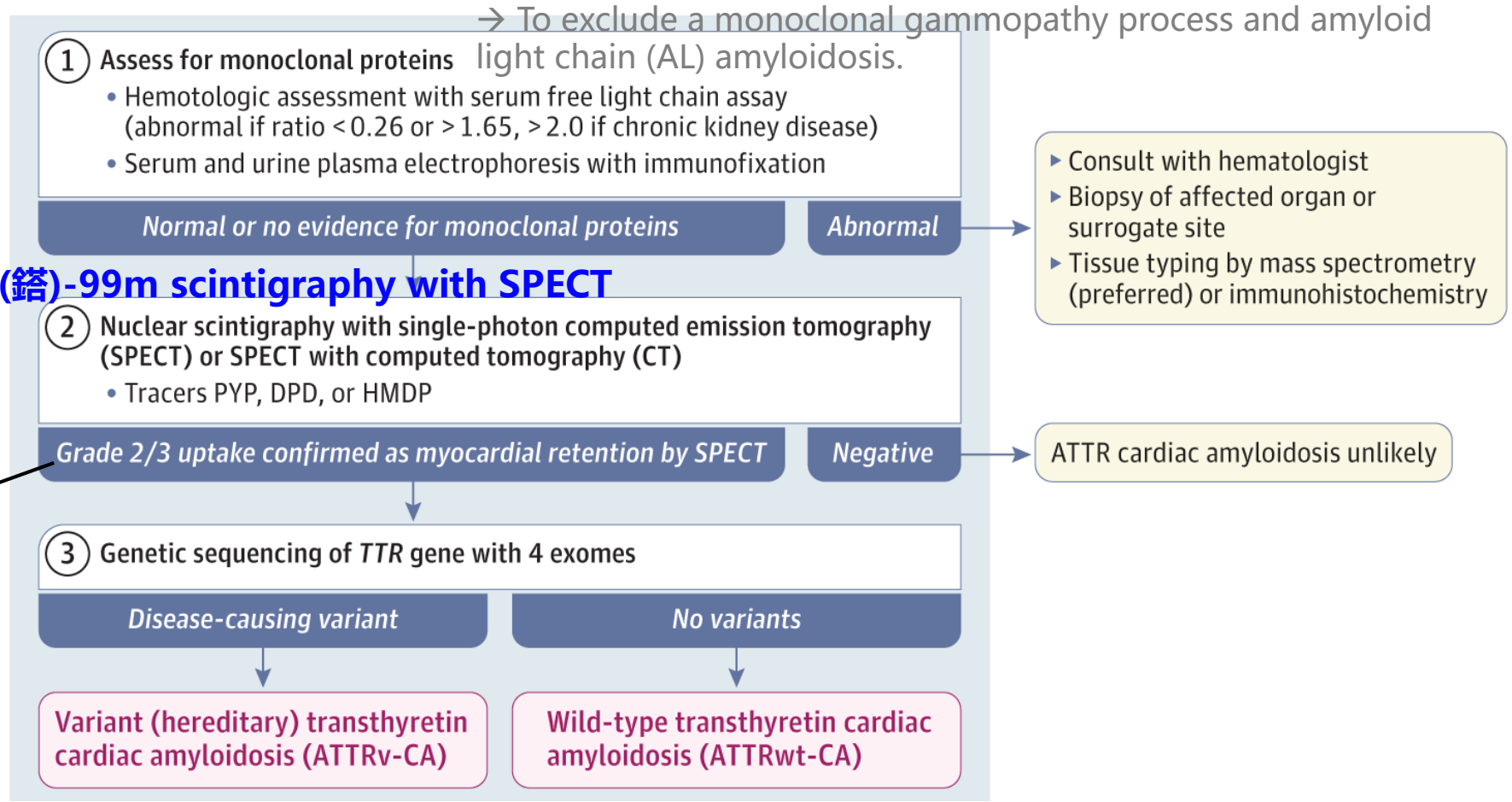
Diagnosis of ATTR Cardiomyopathy

Invasive diagnostic test:
→ **Endomyocardial biopsy**

- Presence of Congo red staining
- Laser capture tandem mass spectrometry demonstrating TTR protein in amyloid deposits

→ **Carries risk of myocardial perforation/tamponade, arrhythmia, and vascular access complication.**

Noninvasive diagnostic test:



Grade	Myocardial Tc-99m Uptake
0	No uptake
1	Uptake < Bone (Ribs)
2	Uptake = Bone (Ribs)
3	Uptake ≥ Bone (Ribs)

PYP: pyrophosphate
DPD: 3,3-diphosphono-1,2-propanodicarboxylic acid
HMDP: hydroxymethylene diphosphonate

JAMA. 2024;331(9):778-791. *J Nucl Cardiol.* 2021;28(2):604-609.

Prognosis

Table 5. Staging Systems for ATTR Cardiac Amyloidosis

Staging system	Staging criteria	Median survival[a]		
		Stage 1 (>5 years)	Stage 2 (3-5 years)	Stage 3 (1-3 years)
Scoring		0 Criteria for Mayo or NAC, score 1 to 3 for Columbia	1 Criterion for Mayo or NAC, score 4 to 6 for Columbia	2 Criteria for Mayo or NAC, score 7 to 9 for Columbia
Mayo Clinic (wild-type ATTR)[33]	Troponin *t* >0.05 ng/mL NT-proBNP >3000 pg/mL	66 mo	40 mo	20 mo
National Amyloidosis Centre (NAC) (wild-type and variant ATTR)[38]	eGFR <45 mL/min/1.73 m2 NT-proBNP >3000 pg/mL	69 mo	47 mo	24 mo
Columbia University (wild-type and variant ATTR)[58]	Mayo or NAC score (0 to 2 points) Daily dose of furosemide equivalents: 0 mg/kg (0 points), >0-0.5 mg/kg (1 point), >0.5-1 mg/kg (2 points), and >1 mg/kg (3 points) NYHA class I-IV (1 to 4 points)	91 mo	36 mo (Mayo) and 39 mo (NAC)	20 mo

Abbreviations: ATTR, amyloidosis from transthyretin; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association.

[a] The Columbia University score ranges from 1 to 9.

General management for ATTR-CM

Heart failure

- Loop diuretics: **furosemide** (< 1 mg/kg/d)
  - Patients with intestinal wall edema (poor absorption of furosemide): torsemide or bumetanide (1 mg of bumetanide=20 mg of torsemide =40 mg of furosemide)
  - Patients with persistent volume overload who do not respond to loop diuretics: combination of loop and thiazide diuretics
- Mineralocorticoid receptor antagonists (MRAs): **spironolactone, eplerenone**
  - independently associated with a reduced risk of mortality.
- β-Blockers: low-dose **bisoprolol** (2.5 mg/d or less)
  - may prevent the increased heart rate response associated with declines in stroke volume in patients with ATTR-CM.
- Refractory HF: Palliative care, Transplantation.
  - ATTRwt: heart transplantation
  - ATTRv: combined heart-liver transplantation

JAMA. 2024;331(9):778-791. Cardiac amyloidosis: Treatment and prognosis. In: UpToDate, Connor RF (Ed), Wolters Kluwer. (Accessed on May 15, 2025.)

General management for ATTR-CM

Arrhythmia

- Atrial fibrillation (AF)：the most common sustained arrhythmia observed in patients with ATTR-CM (30-70%).
  - **Anticoagulation**：insufficient data exist for recommending anticoagulation with vitamin K antagonists or direct oral anticoagulants.
  - Rate and rhythm control：focus on improving symptoms.
  - Cardioversion：recommend transesophageal echocardiography(TEE) prior to cardioversion.
- Atrioventricular block (AV block)：second-degree Mobitz type II or third-degree AVB.
  - **Implantable cardioverter defibrillator (ICD)**：**Biventricular pacing** is preferred (reduces mitral regurgitation severity, preserves NYHA class, and preserves ejection fraction in observational cohort data.)

JAMA. 2024;331(9):778-791. Cardiac amyloidosis: Treatment and prognosis. In: UpToDate, Connor RF (Ed), Wolters Kluwer. (Accessed on May 15, 2025.)

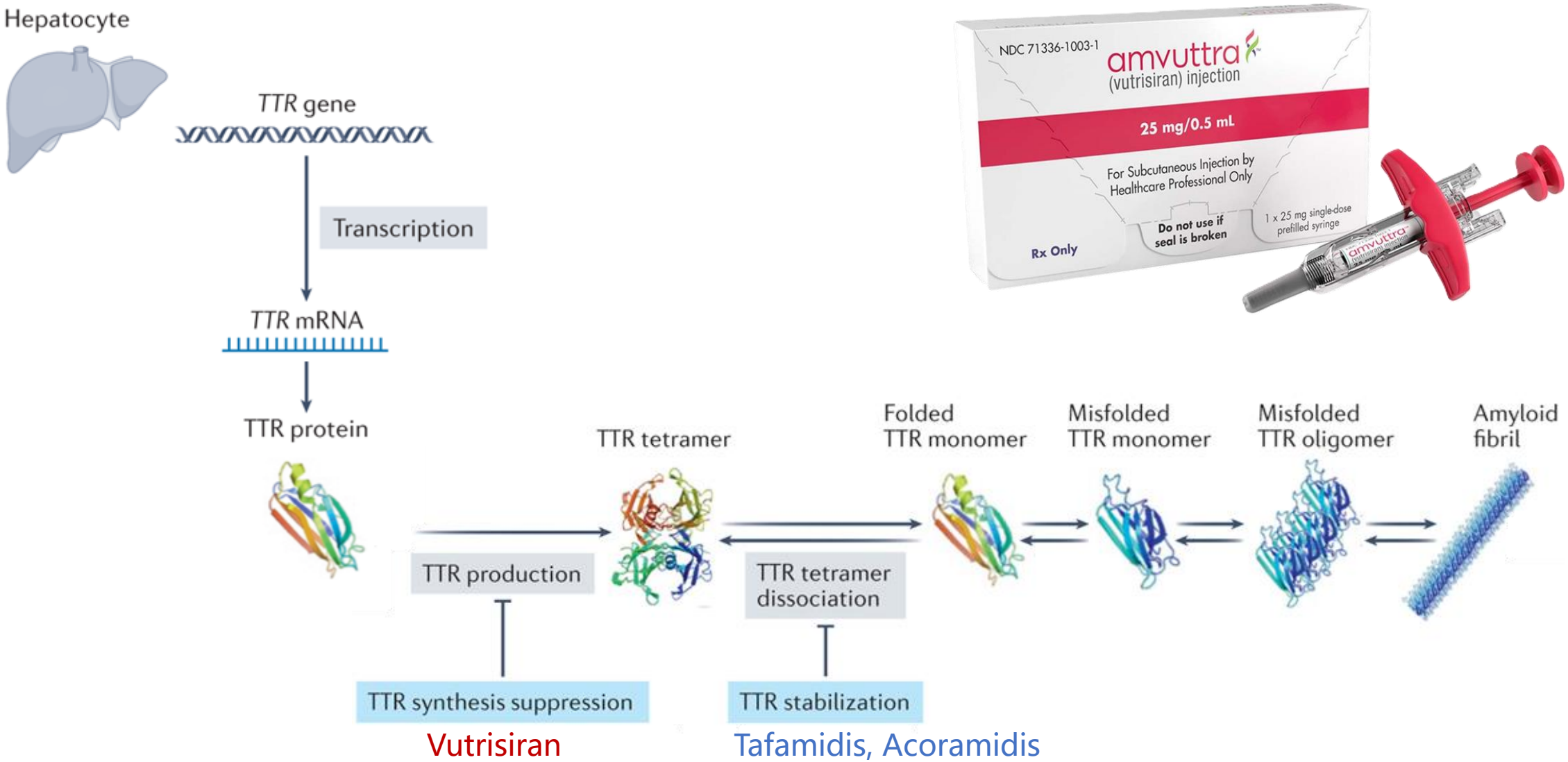
Disease-specific therapy for ATTR-CM

	Vutrisiran (Amvuttra, Alnylam)	**Tafamidis** (Vyndamax, Pfizer) (Vyndaqel, Pfizer)	**Acoramidis** (Attruby, BridgeBio)
Regulatory approval	• hATTR-PN • wtATTR & hATTR-CM	• wtATTR & hATTR-CM	• wtATTR & hATTR-CM
Class & Formulation	• GalNAc-conjugated siRNA • Injection	• Benzoxazole derivative • Capsule	• 3-(3-[3,5-Dimethyl-1Hpyrazol-4-yl]propoxy)-4- fluorobenzoic acid • Tablet
Mechanism of action (MOA)	Binds proteins that form the RISC, with release of passenger strand and antisense strand bound to RISC binds to target TTR mRNA, which is then cleaved by Argonaute2.	Stabilizes TTR tetramer	Stabilizes TTR tetramer
Dose	• **25 mg, SC, Q3M**	• Vyndamax: one **61mg tafamidis** capsule, **PO, QD** • Vyndaqel: four 20mg **tafamidis meglumine** capsules (**total 80mg**), **PO, QD**	• **712mg** (two 356mg tablets), **PO, BID**
Additional medication	vitamin A supplementation daily	None	None
ADR	Arthralgia (11%), limb pain (15%)	None	Diarrhea (12%)

- hATTR-PN: hereditary transthyretin-mediated amyloidosis polyneuropathy
- GalNAc: N-acetylgalactosamine

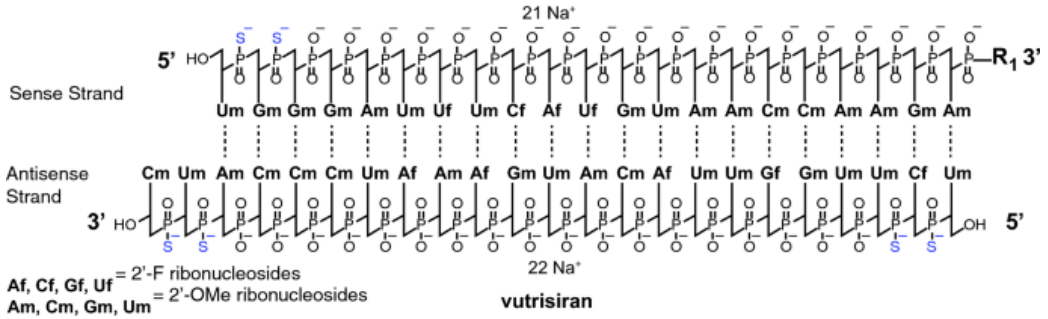
JAMA. 2024;331(9):778-791. Cardiac amyloidosis: Treatment and prognosis. In: UpToDate, Connor RF (Ed), Wolters Kluwer. (Accessed on May 15, 2025.)

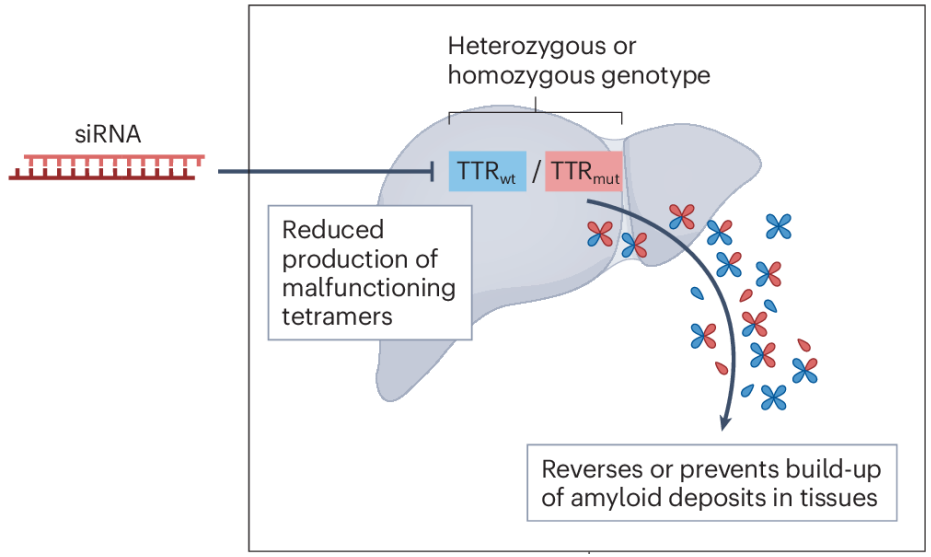
The MOA of Vutrisiran (Amvuttra)

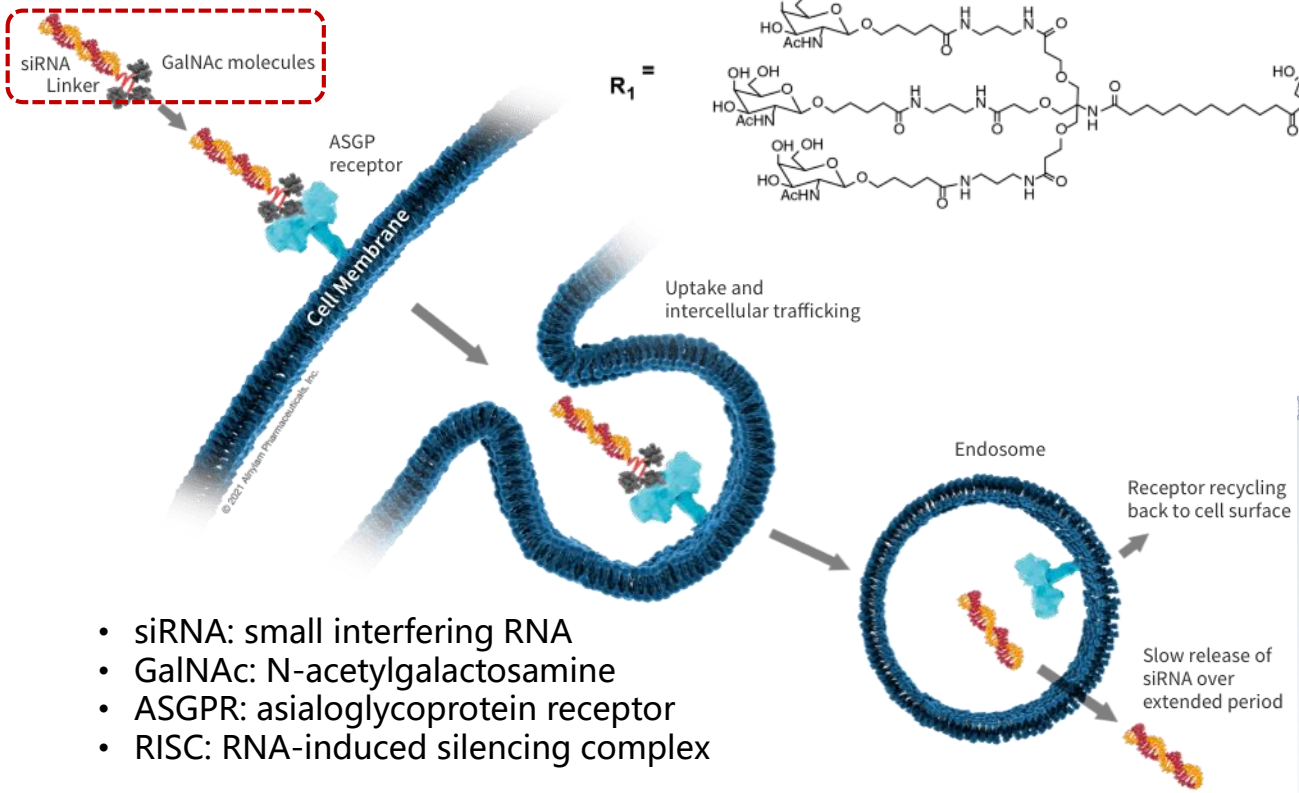


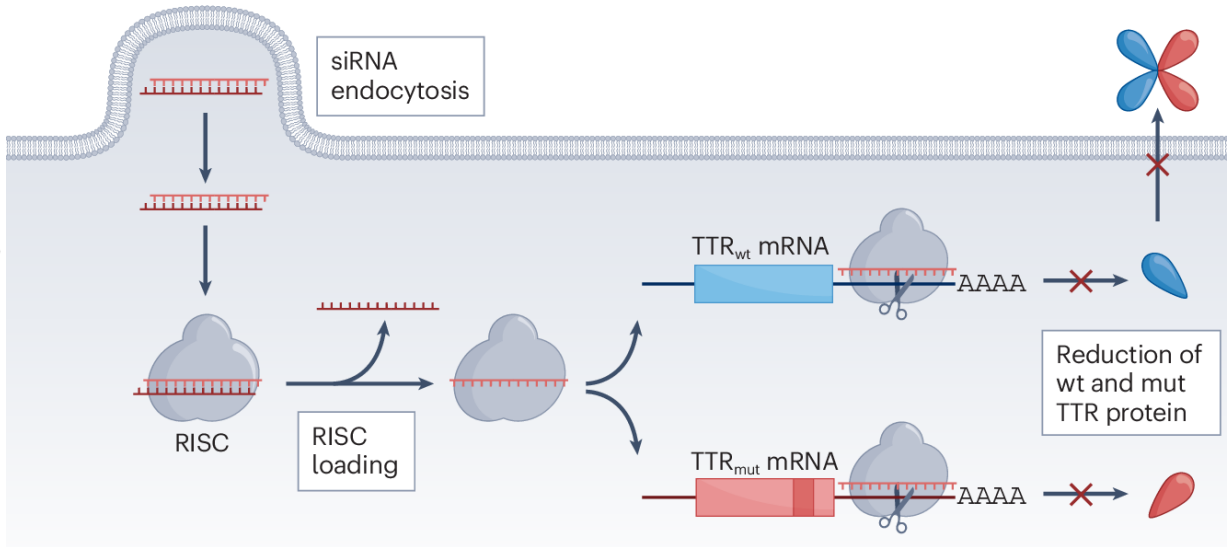
Nat Rev Cardiol. 2022;19(10):655-667.

The MOA of Vutrisiran (Amvuttra)









- siRNA: small interfering RNA
- GalNAc: N-acetylgalactosamine
- ASGPR: asialoglycoprotein receptor
- RISC: RNA-induced silencing complex

Alnylam Pharmaceuticals, Inc. *Nat Rev Drug Discov.* 2024;23(6):421-444. *Drugs.* 2022;82(13):1419-1425.

Disease-specific therapy for ATTR-CM

	Vutrisiran (Amvuttra, Alnylam)	**Tafamidis** (Vyndamax, Pfizer) (Vyndaqel, Pfizer)	**Acoramidis** (Attruby, BridgeBio)
Regulatory approval	• hATTR-PN • wtATTR & hATTR-CM	• wtATTR & hATTR-CM	• wtATTR & hATTR-CM
Class & Formulation	• GalNAc-conjugated siRNA • Injection	• Benzoxazole derivative • Capsule	• 3-(3-[3,5-Dimethyl-1Hpyrazol-4-yl]propoxy)-4- fluorobenzoic acid • Tablet
Mechanism of action (MOA)	Binds proteins that form the RISC, with release of passenger strand and antisense strand bound to RISC binds to target TTR mRNA, which is then cleaved by Argonaute2.	Stabilizes TTR tetramer	Stabilizes TTR tetramer
Dose	• **25 mg, SC, Q3M**	• Vyndamax: one **61mg tafamidis** capsule, **PO, QD** • Vyndaqel: four 20mg **tafamidis meglumine** capsules (**total 80mg**), **PO, QD**	• **712mg** (two 356mg tablets), **PO, BID**
Additional medication	vitamin A supplementation daily	None	None
ADR	Arthralgia (11%), limb pain (15%)	None	Diarrhea (12%)

- hATTR-PN: hereditary transthyretin-mediated amyloidosis polyneuropathy
- GalNAc: N-acetylgalactosamine

JAMA. 2024;331(9):778-791. Cardiac amyloidosis: Treatment and prognosis. In: UpToDate, Connor RF (Ed), Wolters Kluwer. (Accessed on May 15, 2025.)

Methods

HELIOS-B trial

- **Phase 3**
- **Double-blind**
- **Randomized**
- **Placebo-controlled**
- **International & multicenter：87 sites in 26 countries**
  - USA, Canada, Argentina, Peru
  - UK, Ireland, Scotland
  - Sweden, Denmark, Portugal, Netherlands, France, Spain, Germany, Czech Republic, Austria, Belgium, Latvia, Lithuania, Hungary, Croatia, Norway, Poland
  - Australia
  - Israel, Georgia
  - Japan, Republic of Korea

Inclusion criteria

- *Age*
  - 18-85 years

Inclusion criteria

- *Patient and Disease Characteristics*
  - Documented diagnosis of **ATTRwt-CM or ATTRv-CM**
    - **ATTRv-CM (meeting all of the following criteria)**
      a. Documentation of a **TTR pathogenic variant** consistent with ATTRv.
      b. Evidence of cardiac involvement by echocardiography with an **end-diastolic interventricular septal wall thickness >12 mm** (based on central echocardiogram reading at screening).
      c. Amyloid deposits in cardiac or non-cardiac tissue (e.g., fat pad aspirate, salivary gland, median nerve connective sheath) confirmed by **Congo Red (or equivalent) staining** OR **99mTc scintigraphy (DPD-Tc, PYP-Tc, or HMDP) with Grade 2 or 3 cardiac uptake, if MGUS has been excluded.**
      d. If the patient has evidence of MGUS based on serum and urine protein electrophoresis and serum free light chains, documentation of **TTR protein in tissue with immunohistochemistry (IHC) or mass spectrometry is required**.

MGUS: monoclonal gammopathy of undetermined significance

Inclusion criteria

- *Patient and Disease Characteristics*
  - Documented diagnosis of **ATTRwt-CM or ATTRv-CM**
    - **ATTRwt-CM (meeting all of the following criteria)**
      a. Documentation of **absence of pathogenic TTR variant**.
      b. Evidence of cardiac involvement by echocardiography with an **end-diastolic interventricular septal wall thickness >12 mm** (based on central echocardiogram reading at screening).
      c. Amyloid deposits in cardiac tissue with TTR protein identification by **IHC, mass spectrometry**, OR **99mTc scintigraphy (DPD-Tc, PYP-Tc, or HMDP) with Grade 2 or 3 cardiac uptake, if MGUS has been excluded.**
      d. If the patient has evidence of MGUS based on serum and urine protein electrophoresis and serum free light chains, the following is required: documentation of TTR protein in cardiac tissue with IHC or mass spectrometry; OR documentation of TTR protein in non-cardiac tissue (e.g., fat pad aspirate, salivary gland, median nerve connective sheath) with IHC or mass spectrometry AND Grade 2 or 3 cardiac uptake on 99mTc scintigraphy per inclusion criterion 2c.

MGUS: monoclonal gammopathy of undetermined significance

Inclusion criteria

- *Patient and Disease Characteristics*
  - Medical history of **heart failure (HF) with at least 1 prior hospitalization for HF** (not due to arrhythmia or a conduction system disturbance treated with a permanent pacemaker) OR **clinical evidence of HF (with or without hospitalization) manifested by signs and symptoms of volume overload or elevated intracardiac pressures** (e.g., elevated jugular venous pressure, shortness of breath or signs of pulmonary congestion on X-ray or auscultation, peripheral edema) **that requires treatment with a diuretic**.

Inclusion criteria

- *Patient and Disease Characteristics*
  - Patient meets 1 of the following criteria:
    a. **Tafamidis naïve and not actively planning to commence treatment with tafamidis during the first 12 months following randomization** (per exclusion criterion 7) (note: in addition to patients who have never taken tafamidis, those who have previously been on tafamidis and have not received any tafamidis for at least 30 days before the screening visit will be considered tafamidis naïve for the purposes of this study)
    b. **On tafamidis** (note: must be on-label use of commercial tafamidis per an approved cardiomyopathy indication and dose in the country of use).

Inclusion criteria

- *Patient and Disease Characteristics*
  - Patient is clinically stable, with **no cardiovascular (CV)-related hospitalizations within 6 weeks before randomization**, as assessed by the investigator.
  - Screening N-terminal pro–B-type natriuretic peptide (NT-proBNP) **>300 pg/mL and <8500 pg/mL**; in patients with permanent or persistent **atrial fibrillation**, screening NT-proBNP **>600 pg/mL and <8500 pg/mL**.

Inclusion criteria

- *Patient and Disease Characteristics*
  - Able to complete **≥150 m** on the **6-minute walk test (6-MWT)** at screening.
  - Have a **Karnofsky performance status** of **≥60%.**

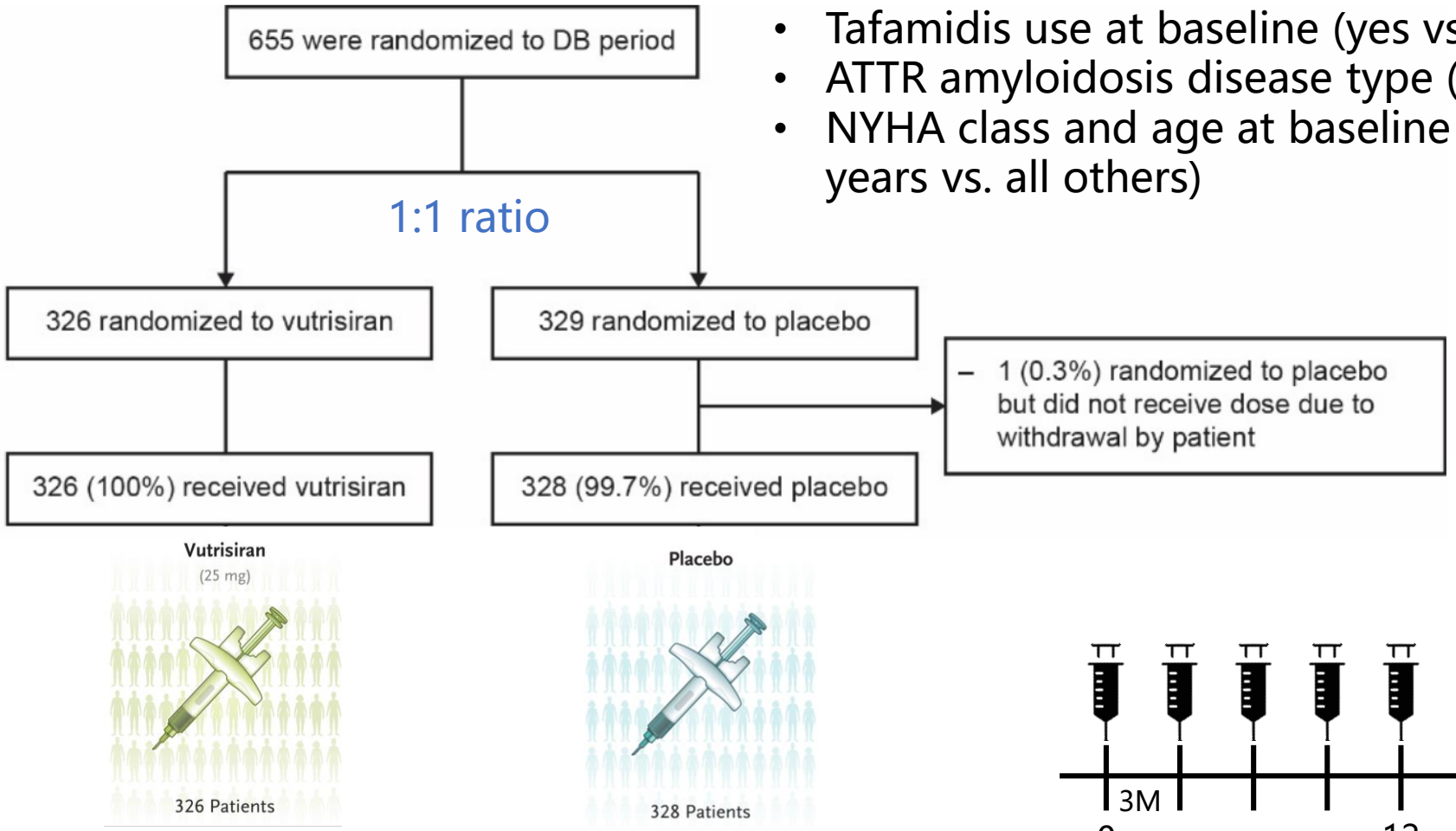
Value	Level of functional capacity	Definition
100	Normal, no complaints, no evidence of disease	Able to carry on normal activity and to work; no special care needed.
90	Able to carry on normal activity, minor signs or symptoms of disease	
80	Normal activity with effort, some signs or symptoms of disease	
70	Cares for self, unable to carry on normal activity or to do active work	Unable to work; able to live at home and care for most personal needs; various degrees of assistance needed.
60	Requires occasional assistance but is able to care for most needs	
50	Requires considerable assistance and frequent medical care	
40	Disabled, requires special care and assistance	Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.
30	Severely disabled, hospitalization is indicated although death is not imminent	
20	Hospitalization is necessary, very sick, active supportive treatment necessary	
10	Moribund, fatal processes progressing rapidly	
0	Dead	

Key Exclusion criteria

- *Disease-specific Conditions*
  - Has known **primary amyloidosis (light chain [AL] amyloidosis)** or **leptomeningeal amyloidosis**.
  - New York Heart Association **(NYHA) Class IV HF**; OR **NYHA Class III HF AND NAC ATTR amyloidosis disease Stage 3** (defined as NT-proBNP >3000 pg/mL and estimated glomerular filtration rate [eGFR]<45 mL/min/1.73 m^2).
  - Has a **polyneuropathy disability score IIIa, IIIb, or IV** (requires cane or stick to walk due to polyneuropathy, or is wheelchair bound) at the screening visit.
- *Laboratory Assessments*
  - Has **eGFR <30** mL/min/1.73 m^2 (using the modification of diet in renal disease formula) at screening.
- *Medical Conditions*
  - **Other non-TTR cardiomyopathy**, hypertensive cardiomyopathy, cardiomyopathy due to valvular heart disease, or cardiomyopathy due to ischemic heart disease (e.g., prior myocardial infarction with documented history of cardiac enzymes and electrocardiographic changes) that the investigator feels is a significant contributor or the predominant cause of the patient's HF.

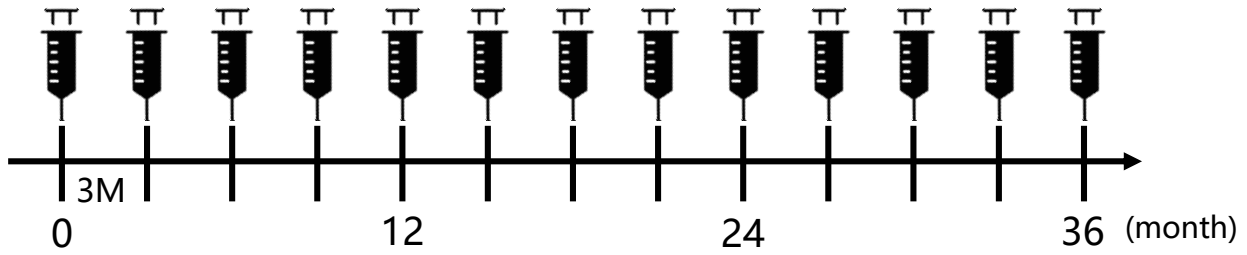
Trial design

DB: double-blind



Randomization:

- Tafamidis use at baseline (yes vs. no)
- ATTR amyloidosis disease type (variant vs. wild type)
- NYHA class and age at baseline (NYHA class I or II and age<75 years vs. all others)

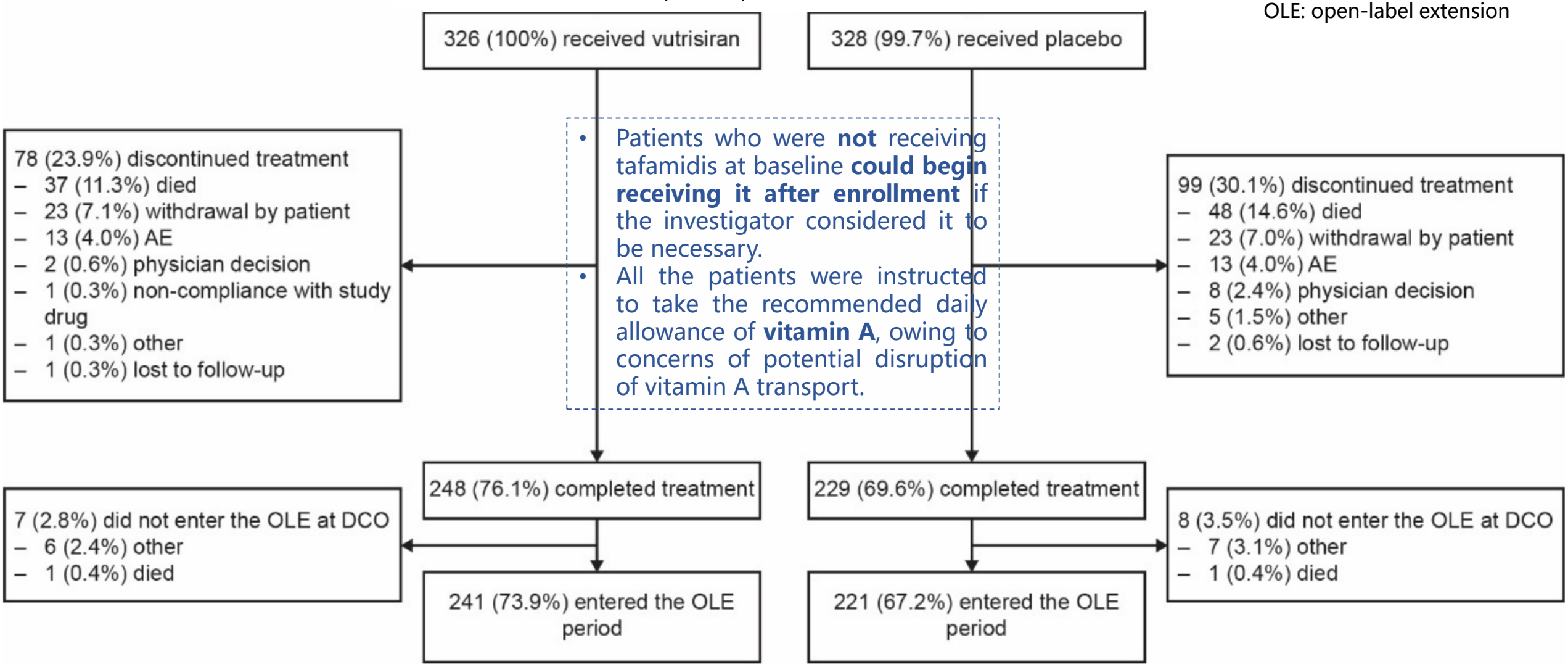


subcutaneously every 12 weeks for up to 36 months.

Trial design

AE: adverse event
DCO: data cut-off
OLE: open-label extension

SC, Q3M, for 36 months



(OLE period for up to 24 months)

End points

Overall population (vutrisiran/placebo **±tafamidis** at baseline)

Monotherapy population (vutrisiran/placebo **-tafamidis** at baseline)

Primary

1. **Death** from any cause* during **DB period (~36 months)**.
2. **Recurrent cardiovascular events** (hospitalizations for cardiovascular causes or urgent visits for heart failure).

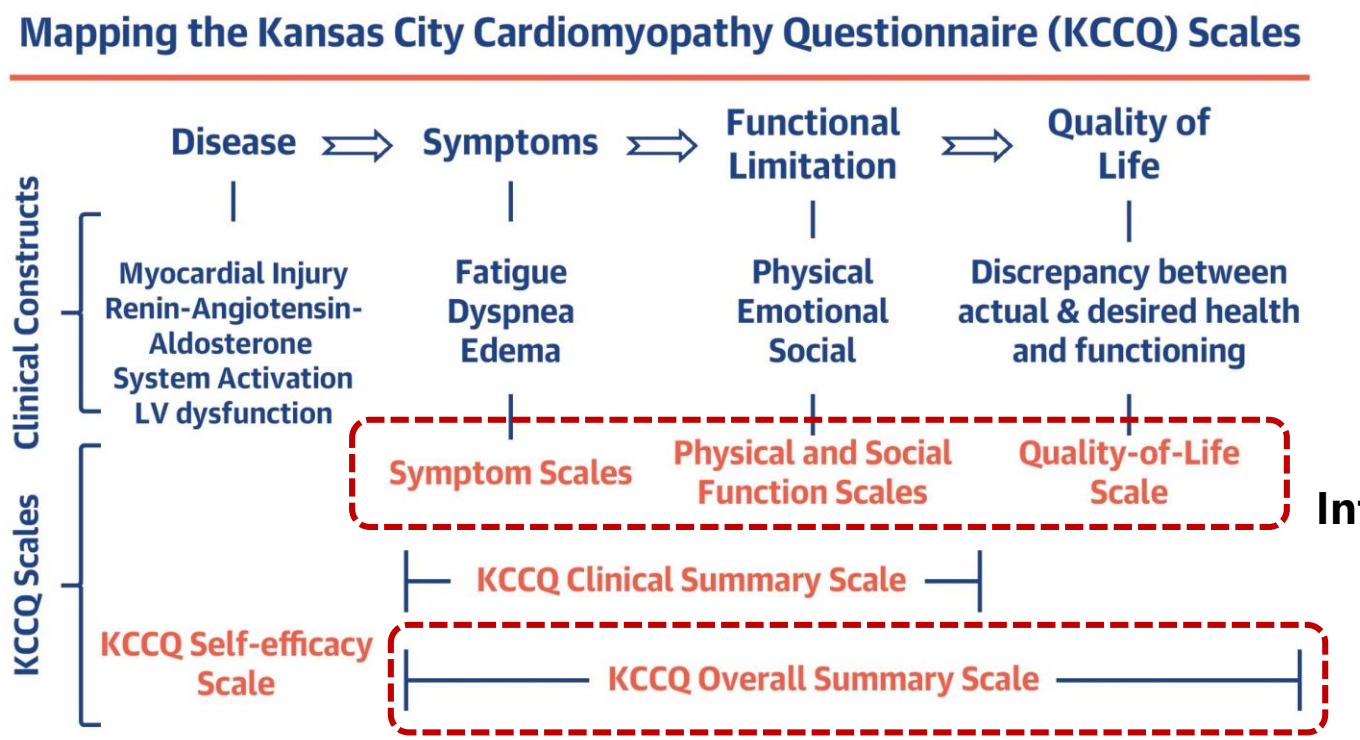
*Heart transplantation or implantation of a left ventricular assist device, or both, were treated as deaths.

Secondary

1. **Death** from any cause through **42 months**.
2. The change from baseline at 30 months in functional capacity (**6-MWT**).
3. Patient-reported health status and health-related quality of life (**KCCQ-OS**).
4. Severity of clinical heart failure symptoms (**NYHA class**).

Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS)

CENTRAL ILLUSTRATION: Conceptual Mapping of the Kansas City Cardiomyopathy Questionnaire to Different Manifestations of Heart Failure



Spertus, J.A. et al. J Am Coll Cardiol. 2020;76(20):2379-90.

Score	Health status
0-24	very poor to poor
25-49	poor to fair
50-74	fair to good
75-100	good to excellent

Interpreting Changes in KCCQ Scores:

- A change of **5 points** is considered to be a **small but clinically important change**.
- Changes of 10 and 20 points are considered moderate-to-large and large-to-very large clinical changes.

J Am Coll Cardiol. 2020;76(20):2379-2390.

End points

Overall population (vutrisiran/placebo **±tafamidis** at baseline)

Monotherapy population (vutrisiran/placebo **-tafamidis** at baseline)

Exploratory
(change from baseline at 30 months)

1. **NT-proBNP** level
2. **Troponin I** level
3. **Peak longitudinal strain** (LV systolic function)
4. Quality of life (EuroQol 5-Dimension 5-Level questionnaire)

Safety
(during DB period)

1. **Adverse events**
2. Clinical laboratory measures
3. Vital signs

Statistical analysis

Sample size and power

With 654 patients, including **60%** of patients in the **monotherapy** population, the power was **approximately 80%** for both primary endpoints in both the overall and monotherapy populations.

Endpoint		Method
Primary	All-cause mortality	• **Modified Andersen–Gill model with a robust variance estimator (LWYY model)** • Sensitivity analysis：Mantel–Haenszel-type stratified win ratio method • Data visualization：Kaplan-Meier curve for time to first CV event or all cause mortality
	Recurrent CV events	
Secondary	All-cause mortality	• **Log-rank test** • Treatment effect：Cox proportional hazards [PH] model. • Survival probability：Kaplan–Meier method with inverse probability of treatment weighting applied.
	6-MWT	• **Mixed-effects model**
	KCCQ-OS	
	NYHA Class	• **Cochran–Mantel–Haenszel method**

Results

Demographic and Clinical Characteristics of the Patients at Baseline

Baseline Characteristics	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
Median age at randomization (range) — **yr**	77.0 (45–85)	76.0 (46–85)	77.5 (46–85)	76.0 (53–85)
Male sex — no. (**%**)	299 (92)	306 (93)	178 (91)	183 (92)

Special consideration related to:		Reference
Sex and gender	• A higher prevalence (69–94%) of ATTR-CM occurs in men compared with women, with a male prevalence of 81–97% reported for ATTRwt-CM and 69–76% reported for ATTRv-CM. • In the APOLLO-B and ATTRibute-CM trials of patients with ATTR-CM, 89% and 90% of patients at baseline were male, respectively.	Eur J Heart Fail 2022;24:2364-6. Circulation 2019;140:16-26. J Am Coll Cardiol 2016;68:161-72. Eur Heart J 2017;38:1895- 904. N Engl J Med 2024;390:132-42. N Engl J Med 2023;389:1553-65.
Age	• Most cases of ATTR-CM occur in patients >65 years of age. • In the APOLLO-B and ATTRibute-CM trials, the median and mean age at baseline were 76 and 77 years, respectively.	Eur J Heart Fail 2022;24:2342-51. N Engl J Med 2024;390:132-42. N Engl J Med 2023;389:1553-65.

Table 1/Table S1/Table S2

Demographic and Clinical Characteristics of the Patients at Baseline

Baseline Characteristics	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
Race — no. (%)				
White	277 (85)	275 (84)	169 (86)	169 (85)
Asian	18 (6)	19 (6)	12 (6)	15 (8)
Black or African American	23 (7)	24 (7)	10 (5)	11 (6)
Other or not reported	8 (2)	10 (3)	5 (3)	4 (2)

Special consideration related to:		Reference
Race or ethnic group	• In the ATTR-ACT, APOLLO-B, and ATTRibute-CM trials, the majority of enrolled patients with ATTR-CM were White (79–88%), with 5–14% and 2–11% of patients being Black and Asian, respectively.	N Engl J Med 2024;390:132-42. N Engl J Med 2023;389:1553-65. N Engl J Med 2018;379:1007-16.

Table 1/Table S1/Table S2

Demographic and Clinical Characteristics of the Patients at Baseline

Baseline Characteristics	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
Wild-type ATTR — no. (%)	289 (89)	289 (88)	173 (88)	174 (87)

Special consideration related to:		Reference
Geography	• In the THAOS registry, most North American and European patients with a predominant cardiac phenotype had ATTRwt. • The TTR V122I variant is the most common variant in the United States. • The TTR V30M variant is endemic in Portugal, Sweden, Brazil, and Japan.	Orphanet J Rare Dis 2022;17:236. Curr Cardiovasc Risk Rep 2021;15:8.
Others	• In the APOLLO-B and ATTRibute-CM trials, 80% and 90% of patients had ATTRwt, respectively.	N Engl J Med 2024;390:132-42. N Engl J Med 2023;389:1553-65.

Table S3. Transthyretin Variants among Patients with ATTRv Enrolled in HELIOS-B.

	Overall Population		Monotherapy Population	
Genotype, n (%)	Vutrisiran (N = 37)	Placebo (N = 39)	Vutrisiran (N = 23)	Placebo (N = 25)
V122I	24 (65)	25 (64)	13 (57)	16 (64)
V30M	4 (11)	2 (5)	4 (17)	2 (8)
T60A	2 (5)	6 (15)	2 (9)	5 (20)
D18E	2 (5)	0	1 (4)	0
D38A	1 (3)	0	1 (4)	0
E89Q	1 (3)	0	1 (4)	0
I84S	1 (3)	0	0	0
S77Y	1 (3)	0	1 (4)	0
S23N	1 (3)	1 (3)	0	0
E89V	0	1 (3)	0	0
I68L	0	2 (5)	0	1 (4)
H88R	0	1 (3)	0	1 (4)
E54L	0	1 (3)	0	0

ATTRv denotes hereditary transthyretin amyloidosis.

Table 1/Table S1/Table S2/Table S3

Demographic and Clinical Characteristics of the Patients at Baseline

Baseline Characteristics	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
Median time since diagnosis of ATTR (range) — **yr**	0.86 (0–11.1)	1.03 (0–10.8)	0.50 (0–8.3)	0.63 (0–6.2)
Tafamidis use at baseline — no. (%)	130 (40)	129 (39)	—	—
Median duration of tafamidis use before start of trial (range) — mo	9.2 (1.1–65.3)	11.3 (1.1–65.5)	—	—

Table S4. Concomitant Medications During the DB Period.

	Overall Population	
	Vutrisiran (N = 326)	Placebo (N = 328)
Tafamidis, n (%)		
Use at baseline	130 (40)	129 (39)
Drop in on monotherapy population during DB period	44/196 (22)	41/199 (21)
Time from study start to initial drop-in dose, months, median (range)	17.7 (6.4–39.1)	17.0 (1.5–33.8)
SGLT2 inhibitor, n (%)		
Use at baseline	10 (3)	11 (3)
Drop in during DB period	102 (31)	114 (35)
Time from study start to initial drop-in dose, months. Median (range)	19.7 (0.1–36.3)	19.6 (0.9–33.3)
Loop diuretics, n (%)		
Use at baseline	261 (80)	259 (79)
Patients with oral diuretic intensification during DB period	155 (48)	183 (56)
New loop diuretics initiated	22 (7)	37 (11)
Dose increase	133 (41)	146 (45)

Loop diuretics include azosemide, bumetanide, furosemide, and torasemide.

Table 1/Table S1/Table S4

Demographic and Clinical Characteristics of the Patients at Baseline

Baseline Characteristics	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
NYHA class — no. (%)				
Ⅰ	49 (15)	35 (11)	15 (8)	12 (6)
Ⅱ	250 (77)	258 (79)	172 (88)	169 (85)
Ⅲ	27 (8)	35 (11)	9 (5)	18 (9)
NAC stage — no. (%) [based on NT-proBNP>3000 pg/mL & eGFR<45 mL/min/1.73 m^2]				
1	208 (64)	229 (70)	113 (58)	138 (69)
2	100 (31)	87 (27)	68 (35)	55 (28)
3	18 (6)	12 (4)	15 (8)	6 (3)
6-MWT, **m**, mean (SD)	372.0 (103.7)	377.1 (96.3)	362.7 (102.7)	372.8 (98.1)
KCCQ-OS, **points**, mean (SD)	73.0 (19.4)	72.3 (19.9)	70.3 (20.2)	69.9 (20.8)

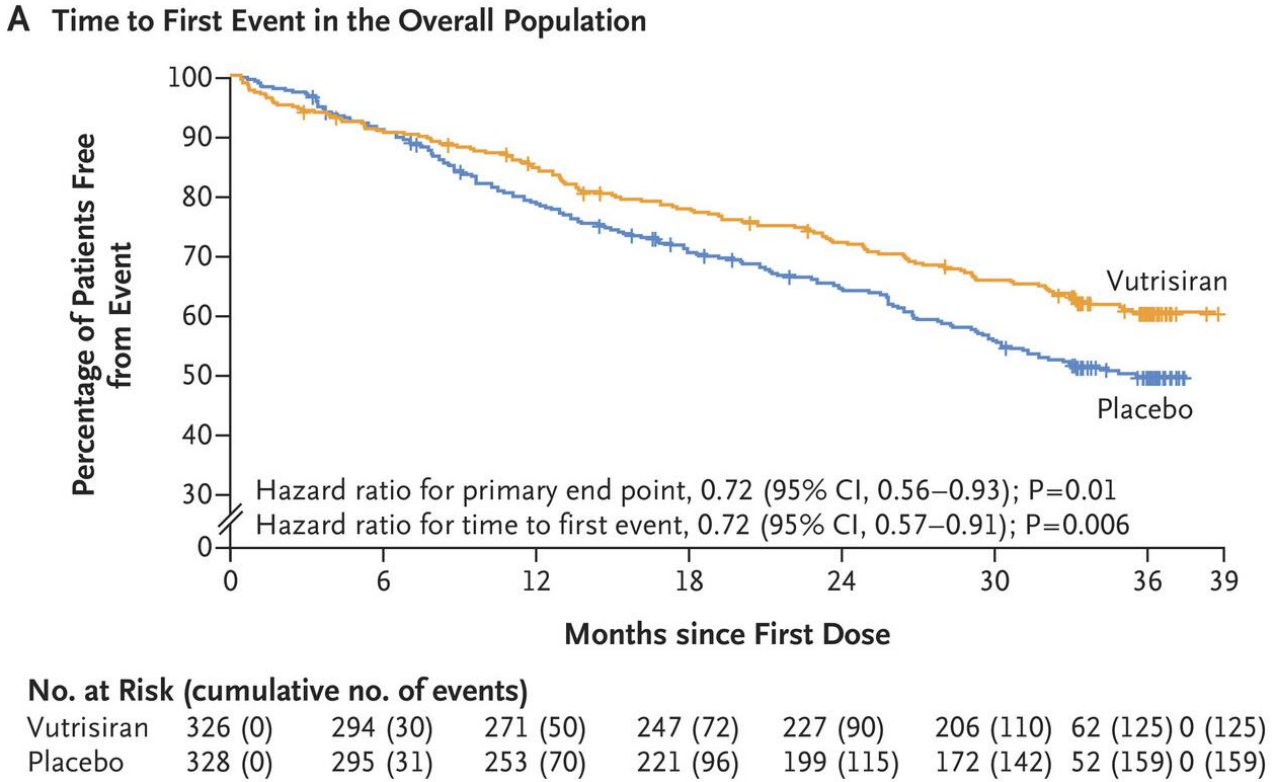
Table 1/Table S1

Demographic and Clinical Characteristics of the Patients at Baseline

Baseline Characteristics	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
Laboratory parameters, **median** (IQR)				
NT-proBNP, pg/mL	2021 (1138–3312)	1801 (1042–3082)	2402 (1322–3868)	1865 (1067–3099)
High-sensitivity troponin I level, pg/mL	71.9 (44.9–115.9)	65.2 (41.1–105.5)	76.3 (48.4–138.8)	62.2 (39.2–105.6)
eGFR, mL/min/1.73 m^2	64 (50–81)	65 (53–81)	64 (50–81)	65 (54–81)
Coexisting conditions, n (**%**)				
Hypertension	185 (57)	187 (57)	107 (55)	111 (56)
Diabetes mellitus	56 (17)	55 (17)	35 (18)	39 (20)
Atrial fibrillation	197 (60)	196 (60)	115 (59)	111 (56)

Table 1/Table S1

In the overall population, treatment with vutrisiran led to a lower risk of death from any cause and recurrent cardiovascular events than placebo.



Primary end point	Overall Population		
	Vutrisiran (N=326)	Placebo (N=328)	Measure of Effect
Death from any cause and recurrent cardiovascular events			Hazard ratio, 0.72 (95% CI, 0.56 to 0.93) P=0.01 [LWYY model]
Patients with at least one event — no. (%)	125 (38)	159 (48)	
Death from any cause	51 (16)	69 (21)	Hazard ratio, 0.69 (95% CI, 0.49 to 0.98) P=0.04 [Cox proportional-hazards model]
Recurrent cardiovascular events	112 (34)	133 (41)	Relative rate ratio, 0.73 (95% CI, 0.61 to 0.88) P=0.001 [Poisson regression model]

Table 2/Figure 1A

In the overall population, treatment with vutrisiran led to a lower risk of death from any cause and recurrent cardiovascular events than placebo.

C Subgroup Analyses of the Primary End Point (overall population)

Subgroup	No. of Patients	Hazard Ratio (95% CI)
Overall	654	0.72 (0.56–0.93)
Age		
<75 yr	257	0.55 (0.35–0.85)
≥75 yr	397	0.81 (0.58–1.11)
Tafamidis use at baseline		
No	395	0.67 (0.49–0.93)
Yes	259	0.79 (0.51–1.21)
ATTR disease type		
Variant	76	0.92 (0.49–1.72)
Wild type	578	0.67 (0.51–0.90)
NYHA class		
I or II	592	0.73 (0.55–0.96)
III	62	0.68 (0.33–1.41)
Baseline NT-proBNP level		
≤2000 pg/ml	342	0.53 (0.35–0.79)
>2000 pg/ml	312	0.80 (0.56–1.13)

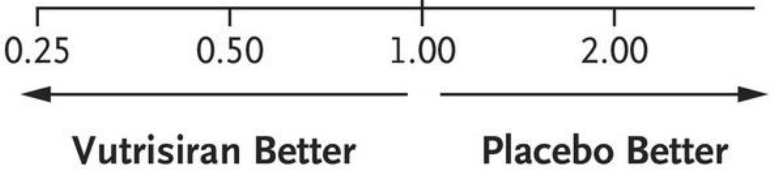
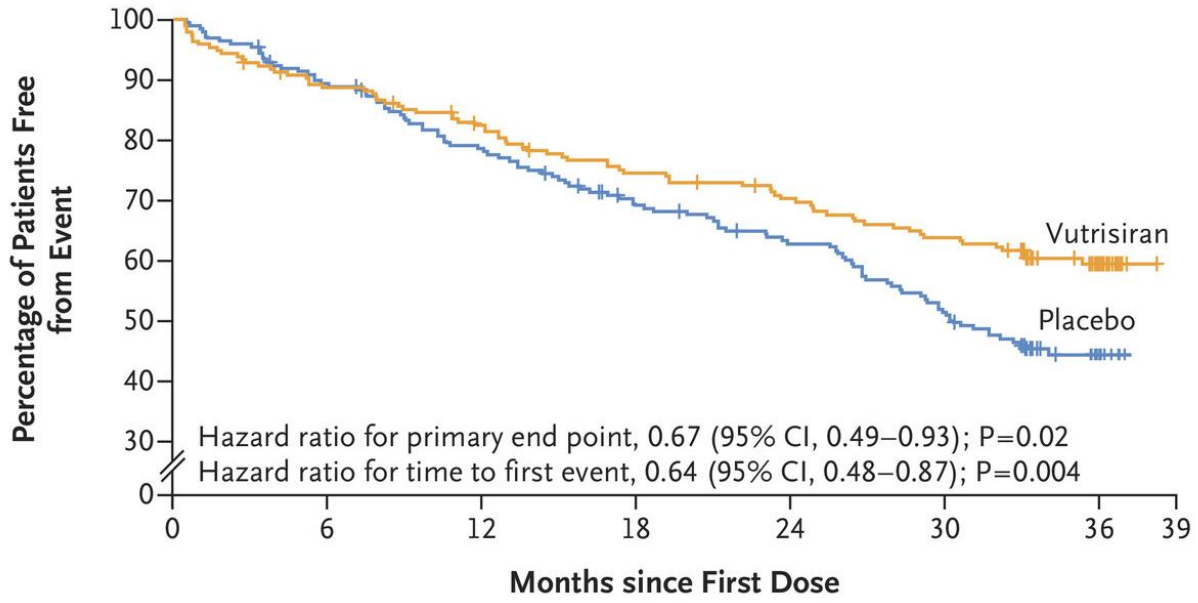


Figure 1C

In the monotherapy population, treatment with vutrisiran led to a lower risk of death from any cause and recurrent cardiovascular events than placebo.

B Time to First Event in the Monotherapy Population



No. at Risk (cumulative no. of events)

Vutrisiran	196 (0)	172 (22)	157 (34)	141 (49)	131 (57)	119 (69)	32 (76)	0 (76)
Placebo	199 (0)	175 (22)	152 (43)	130 (60)	116 (72)	95 (93)	26 (105)	0 (105)

Primary end point	Monotherapy Population		
	Vutrisiran (N=196)	Placebo (N=199)	Measure of Effect
Death from any cause and recurrent cardiovascular events			Hazard ratio, 0.67 (95% CI, 0.49 to 0.93) P=0.02 [LWYY model]
Patients with at least one event — no. (%)	76 (39)	105 (53)	
Death from any cause	36 (18)	46 (23)	Hazard ratio, 0.71 (95% CI, 0.47 to 1.06) P=0.12 [Cox proportional-hazards model]
Recurrent cardiovascular events	66 (34)	87 (44)	Relative rate ratio, 0.68 (95% CI, 0.53 to 0.86) P=0.001 [Poisson regression model]

Table 2/Figure 1B

In the monotherapy population, treatment with vutrisiran led to a lower risk of death from any cause and recurrent cardiovascular events than placebo.

D Subgroup Analyses of the Primary End Point (monotherapy population)

Subgroup	No. of Patients	Hazard Ratio (95% CI)
Overall	395	0.67 (0.49–0.93)
Age		
<75 yr	153	0.53 (0.32–0.88)
≥75 yr	242	0.72 (0.47–1.10)
ATTR disease type		
Variant	48	0.67 (0.31–1.44)
Wild type	347	0.66 (0.45–0.95)
NYHA class		
I or II	368	0.73 (0.53–1.02)
III	27	0.31 (0.09–1.02)
Baseline NT-proBNP level		
≤2000 pg/ml	188	0.50 (0.28–0.92)
>2000 pg/ml	207	0.71 (0.47–1.07)

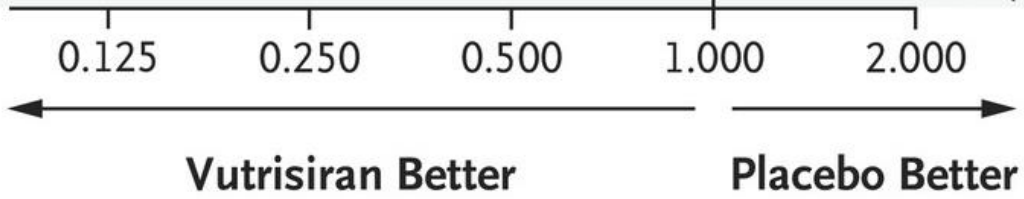
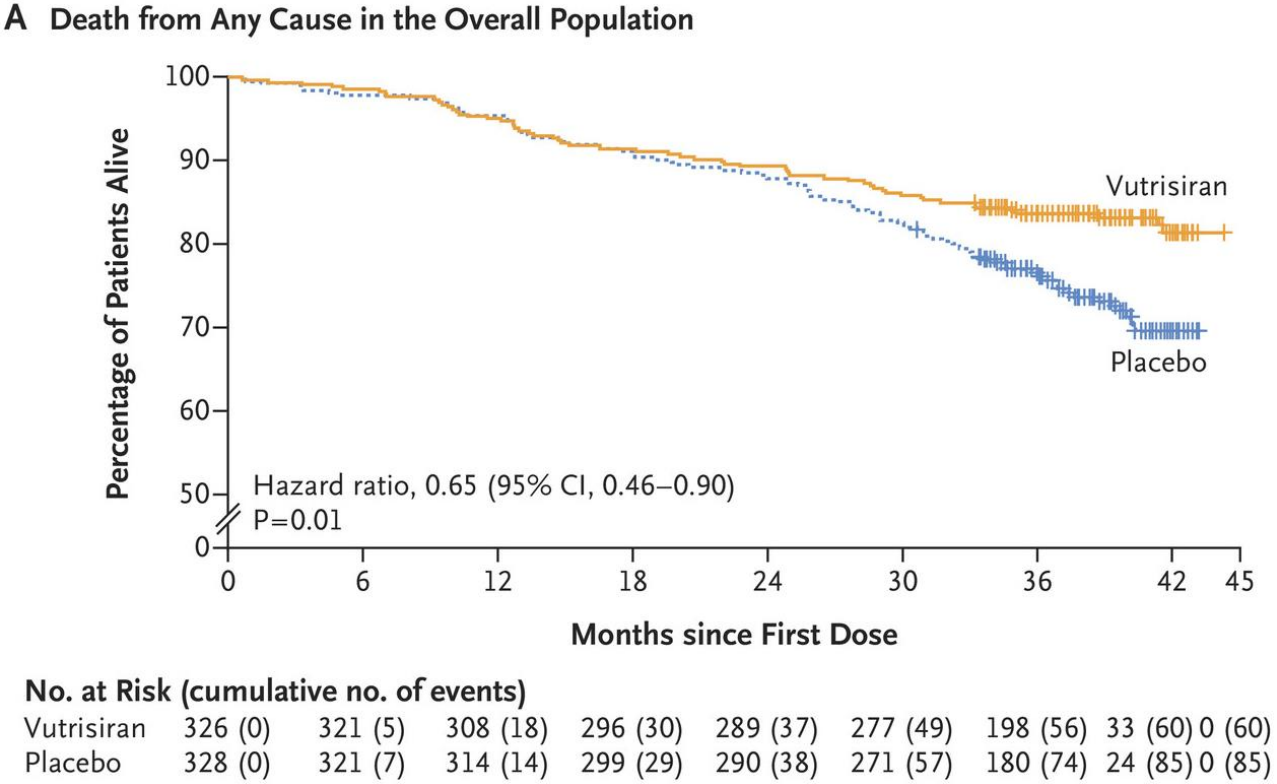


Figure 1D

In the overall population, treatment with vutrisiran resulted in a lower risk of death from any cause through 42 months than placebo.

Secondary end point	Overall Population		
	Vutrisiran (N=326)	Placebo (N=328)	Measure of Effect
Death from any cause through 42 mo			Hazard ratio, 0.65 (95% CI, 0.46 to 0.90) P=0.01
Patients who died — no. (%)	60 (18)	85 (26)	



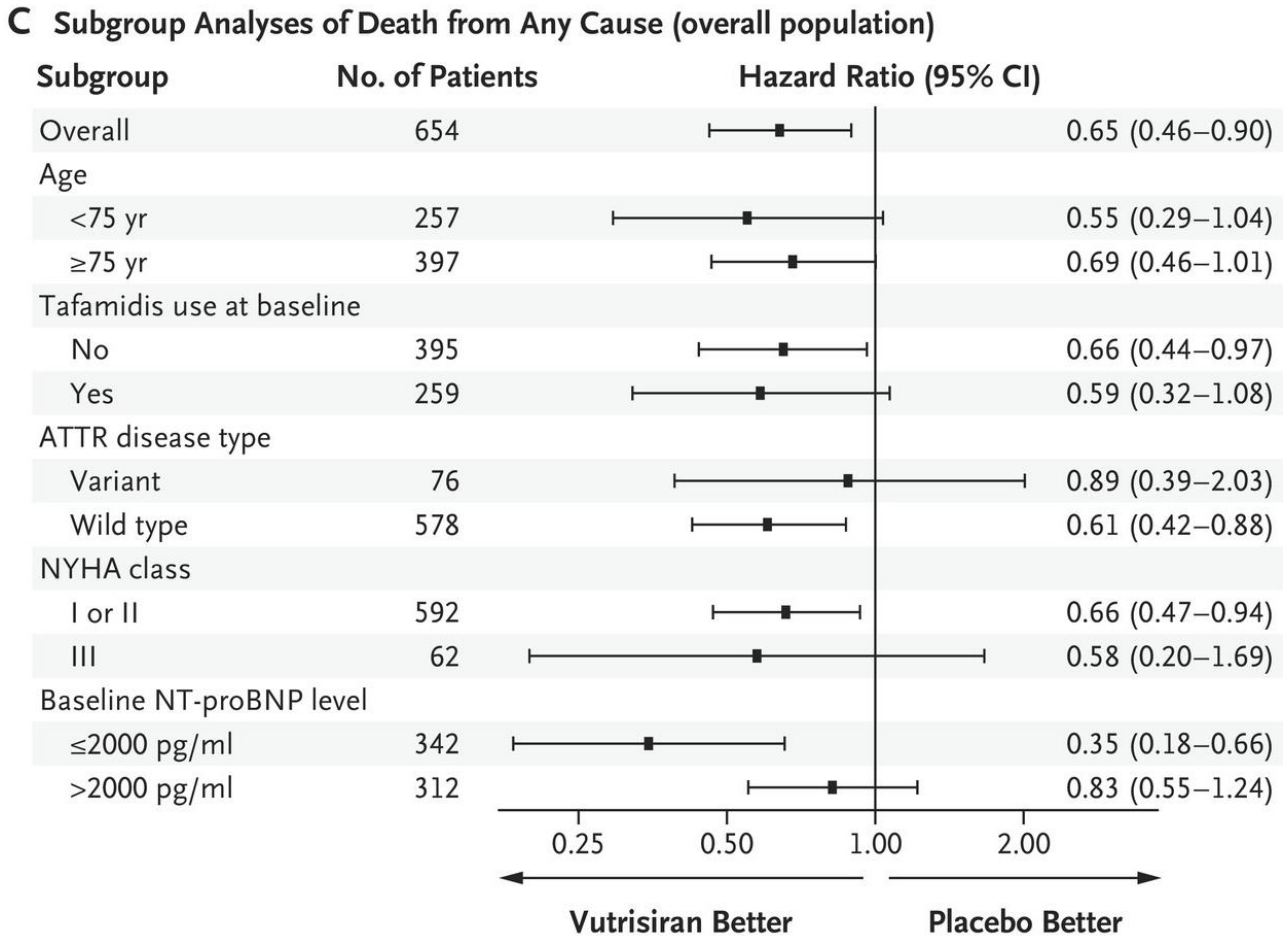
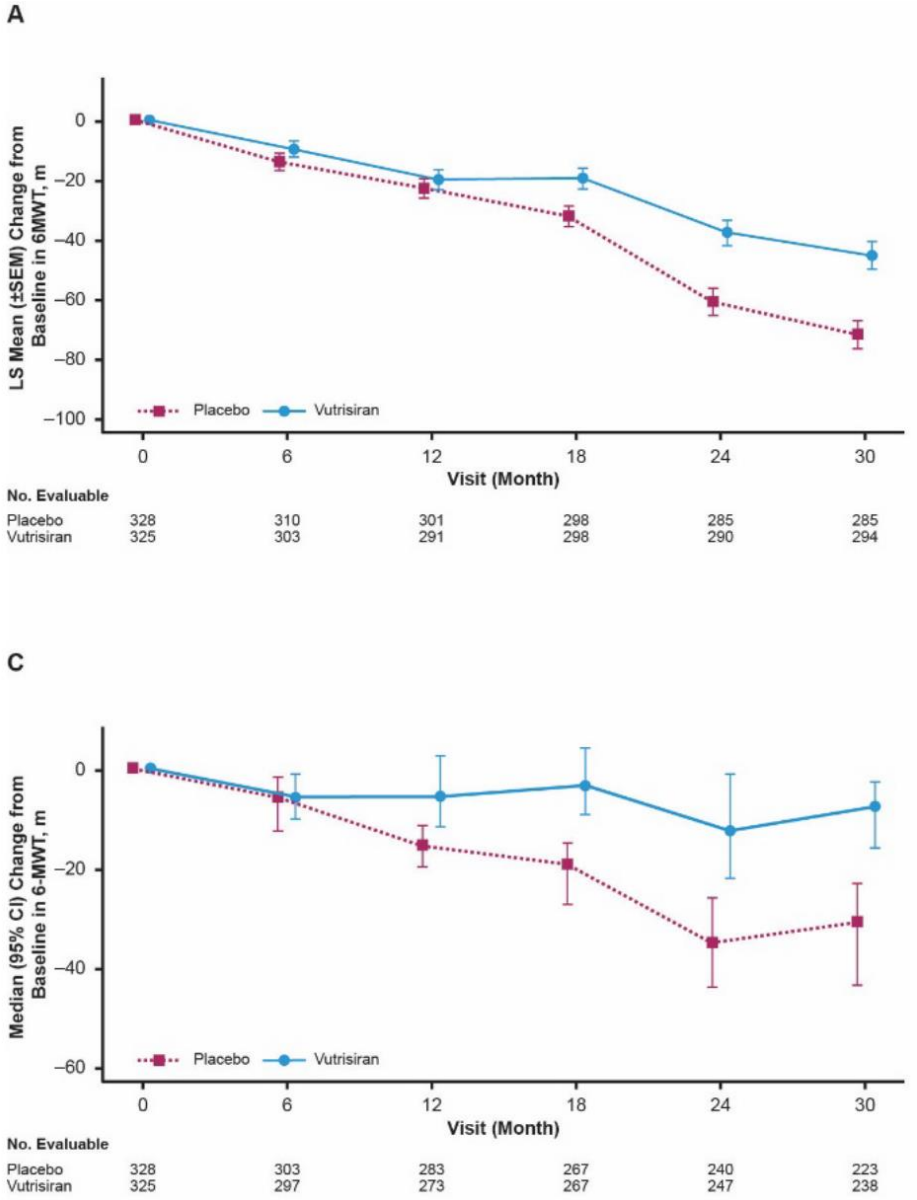


Table 2/Figure 2A/Figure 2C

In the overall population, treatment with vutrisiran resulted in less of a decline in the distance covered on the 6-minute walk test than placebo.

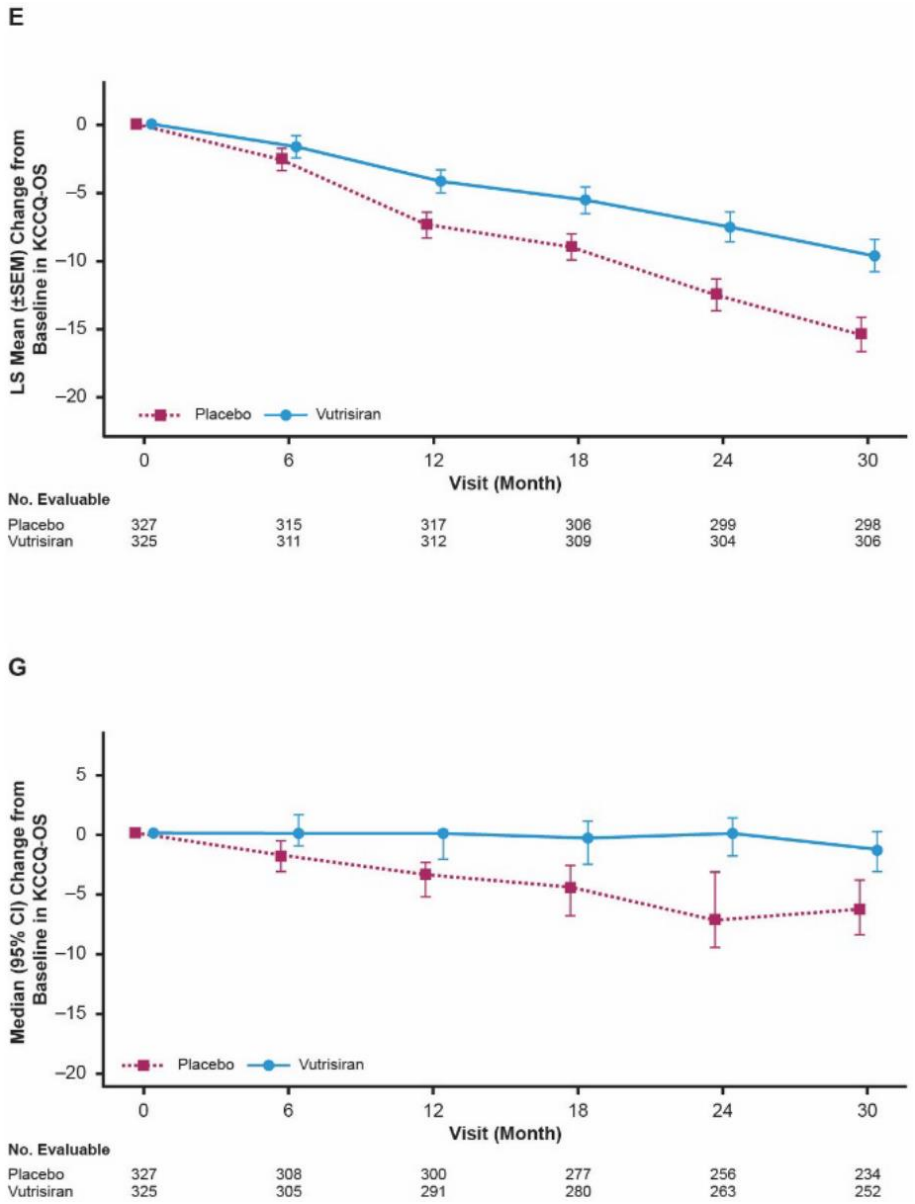


Secondary end point	Overall Population		
	Vutrisiran (N=326)	Placebo (N=328)	Measure of Effect
Least-squares mean change from baseline at 30 mo in distance covered on the 6-min walk test — m	−45.4 (95% CI, −54.5 to −36.3)	−71.9 (95% CI, −81.3 to −62.4)	Difference, 26.5 (95% CI, 13.4 to 39.6) P<0.001
Least-squares mean change from baseline in KCCQ-OS score at 30 mo — points	−9.7 (95% CI, −12.0 to −7.4)	−15.5 (95% CI, −18.0 to −13.0)	Difference, 5.8 (95% CI, 2.4 to 9.2) P<0.001
Improved or stable NYHA class at 30 mo — %	68	61	Difference, 8.7 (95% CI, 1.3 to 16.1) P=0.02

Table 2/Figure S6A/Figure S6C

- LS mean changes impute missing data due to death or inability to walk using the worst 10% of outcomes.
- Median changes reflect the observed results from surviving patients.

In the overall population, treatment with vutrisiran resulted in less of a decline in the KCCQ-OS score than placebo.



Secondary end point	Overall Population		
	Vutrisiran (N=326)	Placebo (N=328)	Measure of Effect
Least-squares mean change from baseline at 30 mo in distance covered on the 6-min walk test — m	−45.4 (95% CI, −54.5 to −36.3)	−71.9 (95% CI, −81.3 to −62.4)	Difference, 26.5 (95% CI, 13.4 to 39.6) P<0.001
Least-squares mean change from baseline in KCCQ-OS score at 30 mo — points	−9.7 (95% CI, −12.0 to −7.4)	−15.5 (95% CI, −18.0 to −13.0)	Difference, 5.8 (95% CI, 2.4 to 9.2) P<0.001
Improved or stable NYHA class at 30 mo — %	68	61	Difference, 8.7 (95% CI, 1.3 to 16.1) P=0.02

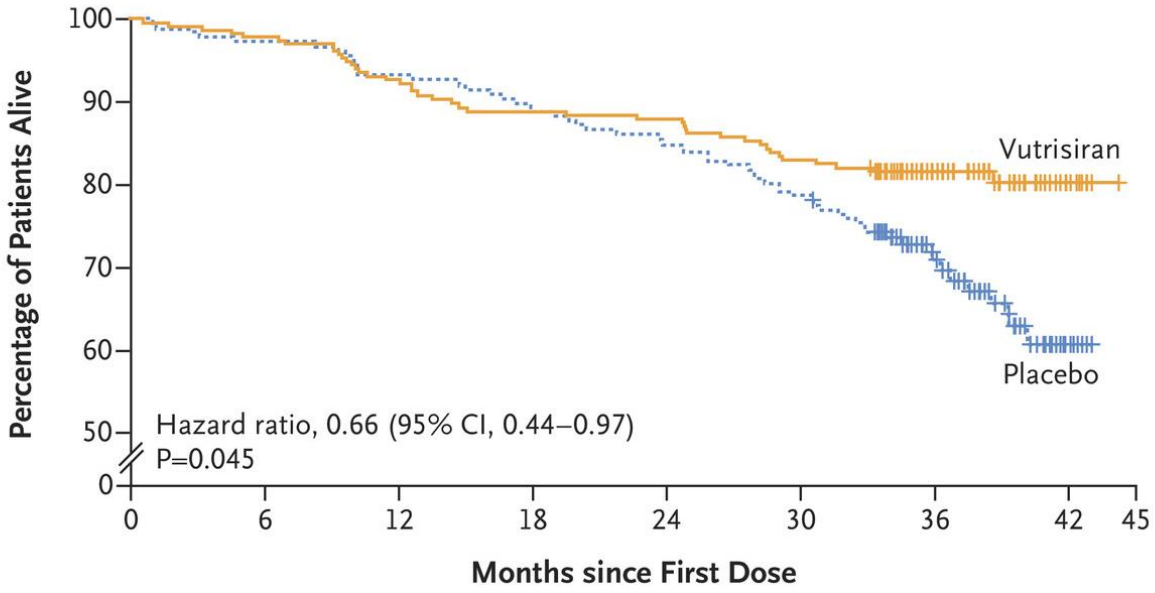
Table 2/Figure S6E/Figure S6G

- LS mean changes impute missing data due to death or inability to walk using the worst 10% of outcomes.
- Median changes reflect the observed results from surviving patients.

In the monotherapy population, treatment with vutrisiran resulted in a lower risk of death from any cause through 42 months than placebo.

Secondary end point	Monotherapy Population		
	Vutrisiran (N=196)	Placebo (N=199)	Measure of Effect
Death from any cause through 42 mo			Hazard ratio, 0.66 (95% CI, 0.44 to 0.97) P=0.045
Patients who died — no. (%)	43 (22)	58 (29)	

B Death from Any Cause in the Monotherapy Population



No. at Risk (cumulative no. of events)

Vutrisiran	196 (0)	191 (5)	179 (17)	171 (25)	169 (27)	158 (38)	86 (41)	17 (43) 0 (43)
Placebo	199 (0)	194 (5)	188 (11)	180 (19)	172 (27)	160 (39)	79 (51)	16 (58) 0 (58)

D Subgroup Analyses of Death from Any Cause (monotherapy population)

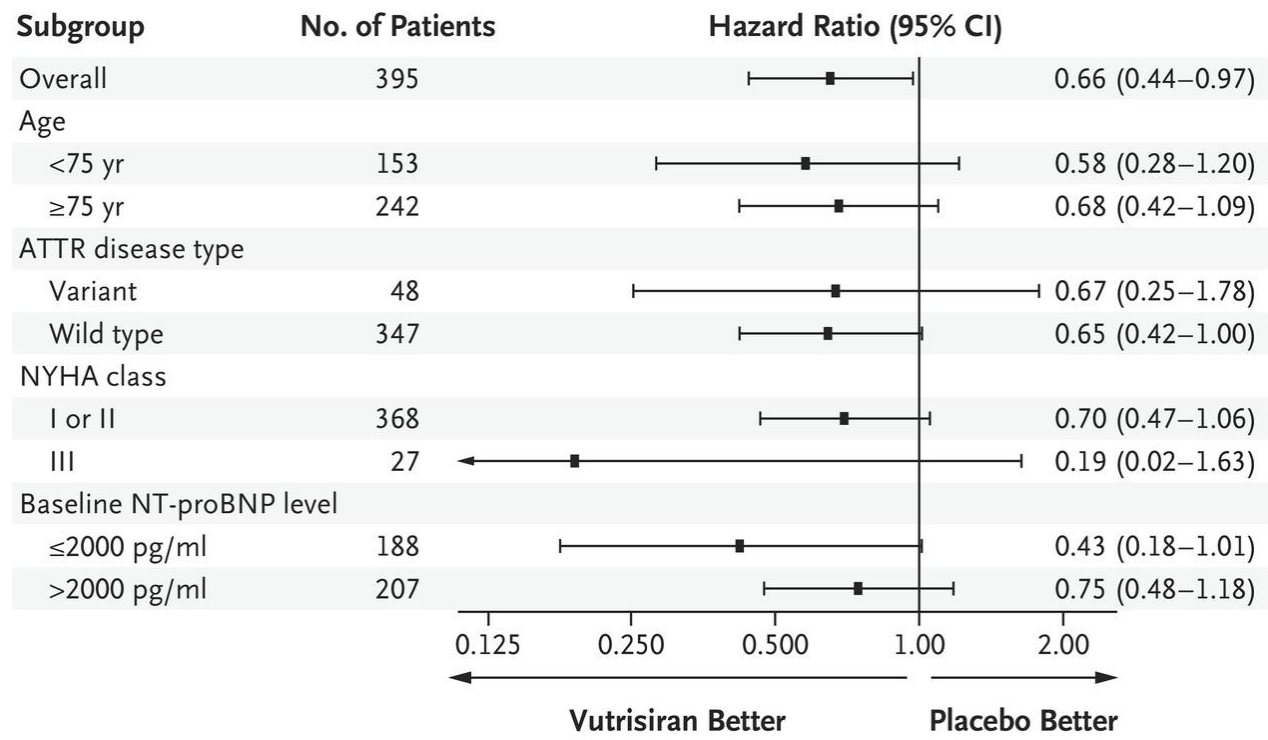
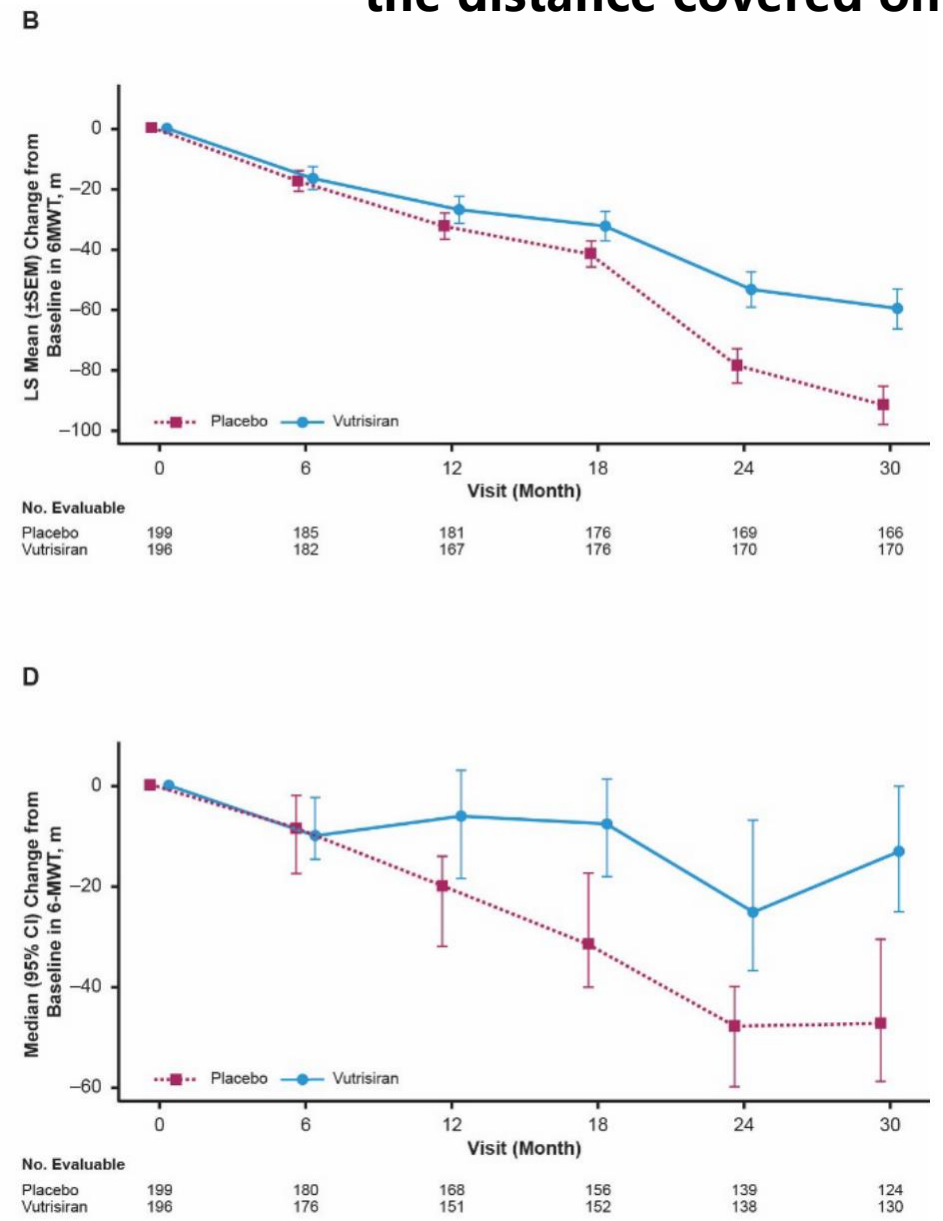


Table 2/Figure 2B/Figure 2D

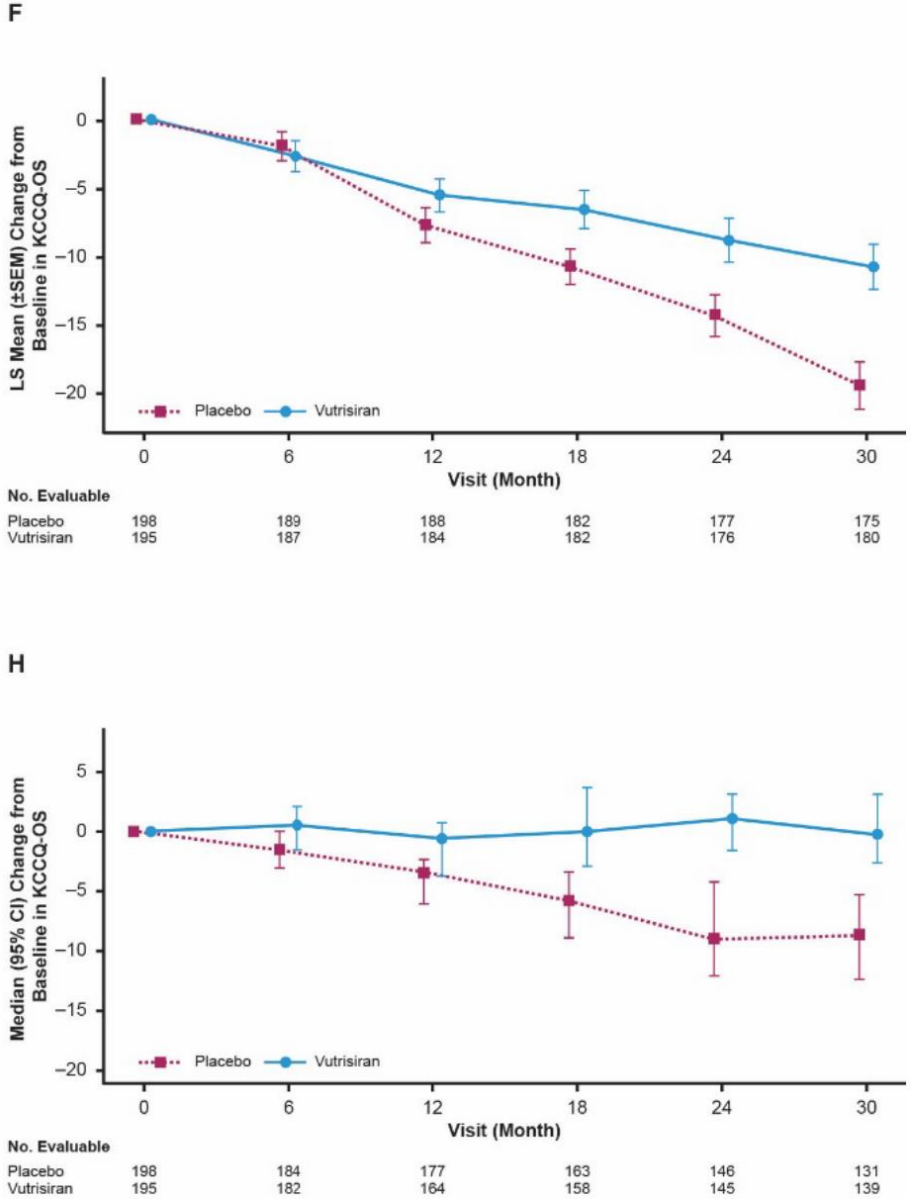
In the monotherapy population, treatment with vutrisiran resulted in less of a decline in the distance covered on the 6-minute walk test than placebo.



Secondary end point	Monotherapy Population		
	Vutrisiran (N=196)	Placebo (N=199)	Measure of Effect
Least-squares mean change from baseline at 30 mo in distance covered on the 6-min walk test — m	−59.7 (95% CI, −72.7 to −46.7)	−91.8 (95% CI, −104.4 to −79.2)	Difference, 32.1 (95% CI, 14.0 to 50.2) P<0.001
Least-squares mean change from baseline in KCCQ-OS score at 30 mo — points	−10.8 (95% CI, −14.1 to −7.5)	−19.5 (95% CI, −22.9 to −16.1)	Difference, 8.7 (95% CI, 4.0 to 13.4) P<0.001
Improved or stable NYHA class at 30 mo — %	66	56	Difference, 12.5 (95% CI, 2.7 to 22.2) P=0.01

Table 2/Figure S6B/Figure S6D

In the monotherapy population, treatment with vutrisiran resulted in less of a decline in the KCCQ-OS score than placebo.



Secondary end point	Monotherapy Population		
	Vutrisiran (N=196)	Placebo (N=199)	Measure of Effect
Least-squares mean change from baseline at 30 mo in distance covered on the 6-min walk test — m	−59.7 (95% CI, −72.7 to −46.7)	−91.8 (95% CI, −104.4 to −79.2)	Difference, 32.1 (95% CI, 14.0 to 50.2) P<0.001
Least-squares mean change from baseline in KCCQ-OS score at 30 mo — points	−10.8 (95% CI, −14.1 to −7.5)	−19.5 (95% CI, −22.9 to −16.1)	Difference, 8.7 (95% CI, 4.0 to 13.4) P<0.001
Improved or stable NYHA class at 30 mo — %	66	56	Difference, 12.5 (95% CI, 2.7 to 22.2) P=0.01

Table 2/Figure S6F/Figure S6H

Treatment with vutrisiran preserved cardiac function

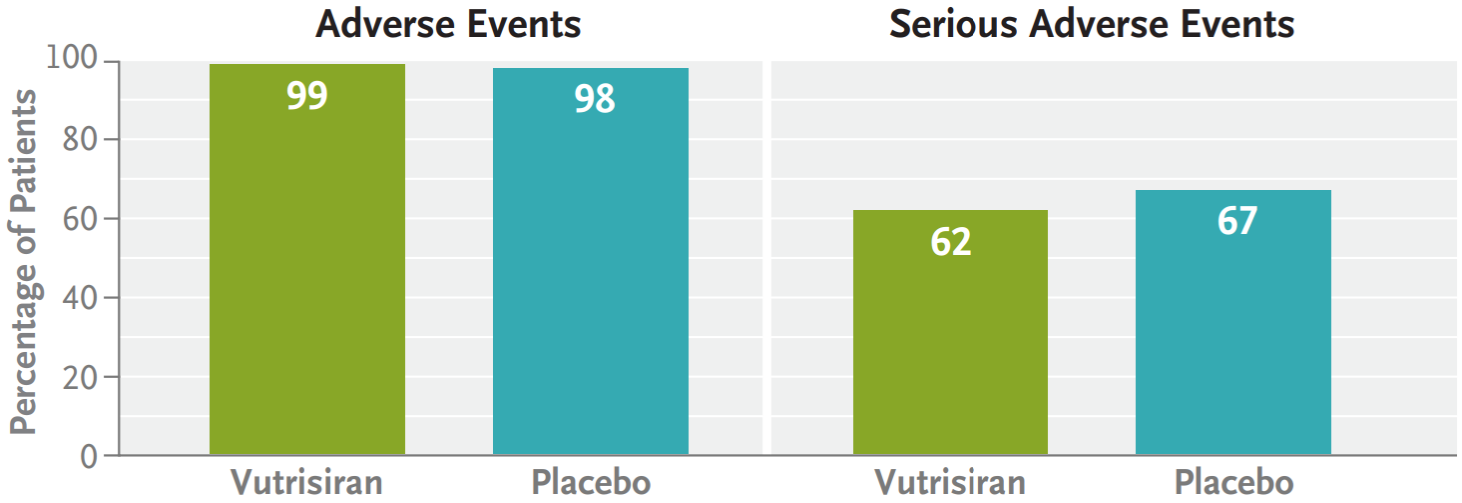
Change from Baseline at 30 Months	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
NT-proBNP fold-change				
Geometric mean (95% CI)	1.19 (1.11, 1.28)	1.75 (1.62, 1.89)	1.30 (1.17, 1.45)	2.28 (2.04, 2.55)
Geometric fold-change ratio (95%CI)	0.68 (0.61, 0.76)		0.57 (0.49, 0.66)	
Troponin I fold-change				
Geometric mean (95% CI)	0.94 (0.88, 1.00)	1.37 (1.28, 1.47)	1.01 (0.92, 1.12)	1.85 (1.68, 2.03)
Geometric fold-change ratio (95%CI)	0.68 (0.62, 0.75)		0.55 (0.48, 0.63)	
Peak longitudinal strain change, %				
LS mean (SEM)	0.95 (0.17)	2.18 (0.19)	1.07 (0.26)	2.37 (0.26)
LS mean difference (95% CI)	-1.23 (-1.73, -0.73)		-1.30 (-2.01, -0.59)	

Table S5

In the overall population, the incidence of adverse events was similar in the two groups.

Table S6. Safety Summary in the Overall Population during the Double-Blind Exposure Period.

Event, n (%)	Vutrisiran (N = 326)	Placebo (N = 328)
At least 1 adverse event	322 (99)	323 (98)
Adverse events occurring in ≥15% of patients in either arm		
Cardiac failure	101 (31)	128 (39)
Covid-19	87 (27)	99 (30)
Atrial fibrillation	69 (21)	68 (21)
Gout	48 (15)	51 (16)
Dyspnea	43 (13)	51 (16)
Fall	42 (13)	69 (21)
Any serious adverse event*	201 (62)	220 (67)
Any severe adverse event*	158 (48)	194 (59)
Serious adverse events occurring in ≥5% of patients in either arm		
Cardiac failure	38 (12)	57 (17)
Atrial fibrillation	26 (8)	20 (6)
Cardiac failure acute	13 (4)	18 (5)
Cardiac adverse events	227 (70)	242 (74)
Cardiac serious adverse events	116 (36)	124 (38)
Any adverse event leading to treatment discontinuation	10 (3)	13 (4)
Any adverse event leading to death†	49 (15)	63 (19)



*Serious adverse events were defined as adverse events that resulted in death, were life-threatening, resulted in inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or clinically significant disability or incapacity, were a congenital anomaly or birth defect, or were important medical events as determined by the investigators.

Table S6

Discussion

Limitations and remaining questions

- Tafamidis was permitted as background therapy in both groups; therefore, the trial did not allow for a randomized comparison of vutrisiran alone with tafamidis alone.
- The trial was not powered to show statistical significance within the subgroup of patients who were taking tafamidis at baseline.
- Most participants were men and were White, but this is consistent with the reported demographic characteristics of patients with ATTR amyloidosis with cardiomyopathy.

Appraisal

Tools

Randomized Controlled Trial Checklist

Risk of Bias 2 (RoB 2) tool

Section A：Is the basic study design **valid** for a randomized controlled trial?

1. Did the study address a clearly formulated research question?

☑ **Yes** ☐ No ☐ Can't tell

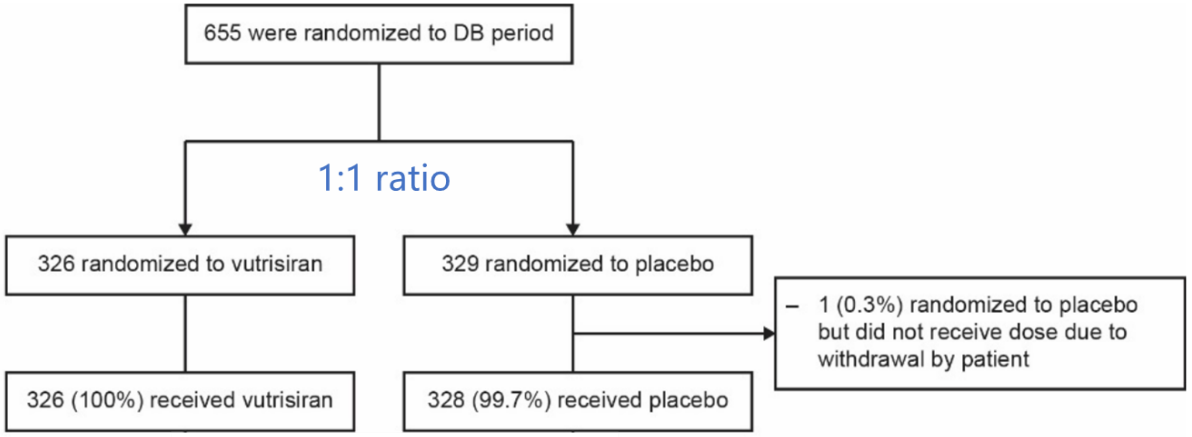
Patient	654 patients with ATTR-CM (both wtATTR and hATTR)
Intervention	Vutrisiran 25 mg, SC, Q3M
Comparison	Placebo, SC, Q3M
Outcome	Primary endpoint： Death from any cause + Recurrent CV events (hospitalizations for cardiovascular causes or urgent visits for heart failure) through 36 months Key secondary endpoints： All-cause mortality at 42 months, 6-minute walk test (6MWT), KCCQ-OS

Section A: Is the basic study design **valid** for a randomized controlled trial?

2. Was the assignment of participants to interventions randomized?

☑ **Yes** ☐ No ☐ Can't tell

- The HELIOS-B trial was an international, phase 3, multicenter, **double-blind**, **randomized**, placebo-controlled trial.



- [*Protocol*] Using **Interactive Response Technology (IRT)**, patients were randomized 1:1 to the vutrisiran or placebo arm. Randomization was stratified by:
  1. Baseline tafamidis use (yes versus no)
  2. ATTR disease type (hATTR versus wtATTR amyloidosis with cardiomyopathy)
  3. NYHA Class I or II and age<75 years versus all other

Section A: Is the basic study design **valid** for a randomized controlled trial?

3. Were all participants who entered the study accounted for at its conclusion?

☑ **Yes** ☐ No ☐ Can't tell

Intention-to-treat (ITT) principle used via **Full Analysis Set (FAS)** :

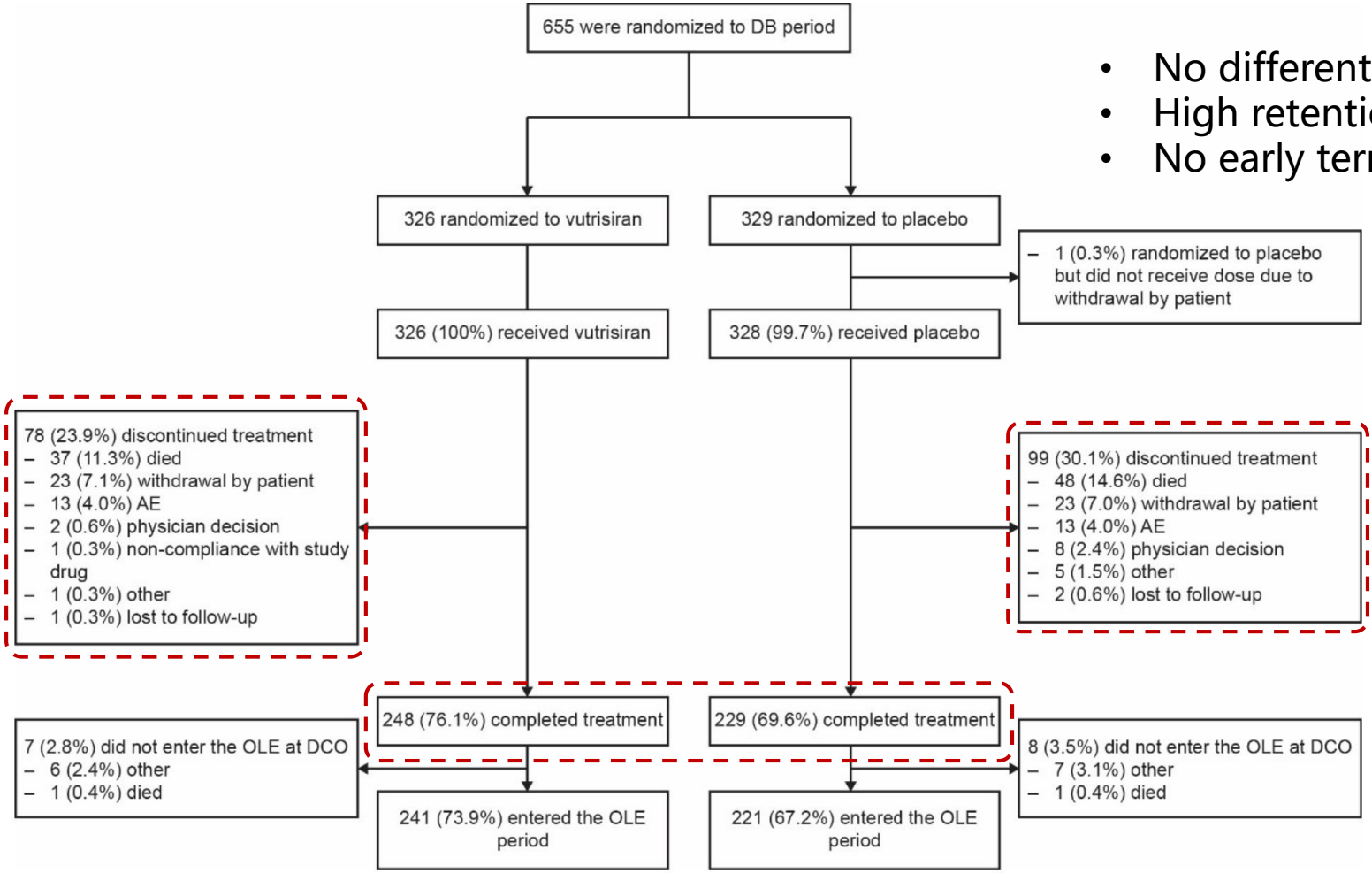
[*Protocol*]

- **Full Analysis Set (FAS)**: All randomized patients who received any amount of study drug. Primary efficacy analyses will be based on the FAS. Patients in the FAS will be analyzed according to the treatment to which they were randomized.
- **Vutrisiran Monotherapy Subgroup FAS (mono-FAS)**: All patients who were not on tafamidis at the study baseline in the FAS. Patients will be analyzed according to the treatment to which they were randomized.

Section A: Is the basic study design **valid** for a randomized controlled trial?

3. Were all participants who entered the study accounted for at its conclusion?

☑ **Yes** ☐ No ☐ Can't tell



- No differential drop-out between study groups
- High retention rate
- No early termination for efficacy

Section A: Is the basic study design **valid** for a randomized controlled trial?

3. Were all participants who entered the study accounted for at its conclusion?

☑ **Yes** ☐ No ☐ Can't tell

[*Protocol*] **Handling of Missing Data**

- No imputation will be done for early dropout patients for the primary analysis of the composite outcome endpoint.
- For longitudinal endpoints including 6-MWT and KCCQ-OS, the primary analyses will be based on **mixed effects model repeated measures (MMRM)** which implicitly impute missing values assuming **missing at random (MAR)**. The only exception is missing due to death and unable to walk due to progression of ATTR amyloidosis (for 6-MWT only).
- For NYHA class, the missing due to death will also be imputed as class IV, and missing due to other reasons will be imputed via multiple imputation.
- Sensitivity analysis of the composite outcome endpoint will be conducted using Multiple Imputation (MI) to handle missing data.
- Sensitivity analyses for 6-MWT and KCCQ-OS will be conducted based on a **pattern mixture model (PMM)** which assume missing not at random (MNAR).
- Since baseline NT-proBNP will be used as a covariate in the statistical models, for one patient with missing baseline NT-proBNP, the baseline value will be imputed with Week 12 visit data.

Section B: Was the study methodologically sound?

4. (a) Were the participants 'blind' to intervention they were given?

☑ **Yes** ☐ No ☐ Can't tell

4. (b) Were the investigators 'blind' to the intervention they were giving to participants?

☑ **Yes** ☐ No ☐ Can't tell

4. (c) Were the people assessing/analyzing outcome/s 'blind' ?

☑ **Yes** ☐ No ☐ Can't tell

[*Supplementary Appendix*] **Blinding**

All site personnel, patients, and Alnylam were blinded to study drug treatment. Vutrisiran and placebo were **packaged identically** with the outside of the prefilled syringe barrel masked to hide the identity of the study drug. **All study personnel were blinded to any clinical laboratory results**, and patients and their physicians were prohibited from obtaining prealbumin and vitamin A levels, other than the blinded assessments scheduled in the study, unless clinically indicated.

Section B: Was the study methodologically sound?

5. Were the study groups similar at the start of the randomized controlled trial?

☑ **Yes** ☐ No ☐ Can't tell

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Overall Population		Monotherapy Population	
	Vutrisiran (N=326)	Placebo (N=328)	Vutrisiran (N=196)	Placebo (N=199)
Median age at randomization (range) — yr	77.0 (45–85)	76.0 (46–85)	77.5 (46–85)	76.0 (53–85)
Male sex — no. (%)	299 (92)	306 (93)	178 (91)	183 (92)
Race — no. (%)†				
White	277 (85)	275 (84)	169 (86)	169 (85)
Asian	18 (6)	19 (6)	12 (6)	15 (8)
Black	23 (7)	24 (7)	10 (5)	11 (6)
Other or not reported	8 (2)	10 (3)	5 (3)	4 (2)
Wild-type ATTR — no. (%)	289 (89)	289 (88)	173 (88)	174 (87)
Median time since diagnosis of ATTR (range) — yr	0.86 (0–11.1)	1.03 (0–10.8)	0.50 (0–8.3)	0.63 (0–6.2)
Tafamidis use at baseline — no. (%)	130 (40)	129 (39)	—	—
Median duration of tafamidis use before start of trial (range) — mo	9.2 (1.1–65.3)	11.3 (1.1–65.5)	—	—
NYHA class — no. (%)				
I	49 (15)	35 (11)	15 (8)	12 (6)
II	250 (77)	258 (79)	172 (88)	169 (85)
III	27 (8)	35 (11)	9 (5)	18 (9)
NAC stage — no. (%)‡				
1	208 (64)	229 (70)	113 (58)	138 (69)
2	100 (31)	87 (27)	68 (35)	55 (28)
3	18 (6)	12 (4)	15 (8)	6 (3)
Laboratory values				
Median NT-proBNP level (IQR) — pg/ml	2021 (1138–3312)	1801 (1042–3082)	2402 (1322–3868)	1865 (1067–3099)
Median high-sensitivity troponin I level (IQR) — pg/ml	71.9 (44.9–115.9)	65.2 (41.1–105.5)	76.3 (48.4–138.8)	62.2 (39.2–105.6)

Section B: Was the study methodologically sound?

6. Apart from the experimental intervention, did each study group receive the same level of care (that is, were they treated equally)?

☑ **Yes** ☐ No ☐ Can't tell

- Patients who were not receiving tafamidis at baseline could begin receiving it after enrollment if the investigator considered it to be necessary.
- All the patients were instructed to take the recommended daily allowance of vitamin A, owing to concerns of potential disruption of vitamin A transport (transthyretin is a vitamin A [retinol] carrier).

Table S4. Concomitant Medications During the DB Period.

	Overall Population	
	Vutrisiran (N = 326)	Placebo (N = 328)
Tafamidis, n (%)		
Use at baseline	130 (40)	129 (39)
Drop in on monotherapy population during DB period	44/196 (22)	41/199 (21)
Time from study start to initial drop-in dose, months, median (range)	17.7 (6.4–39.1)	17.0 (1.5–33.8)
SGLT2 inhibitor, n (%)		
Use at baseline	10 (3)	11 (3)
Drop in during DB period	102 (31)	114 (35)
Time from study start to initial drop-in dose, months. Median (range)	19.7 (0.1–36.3)	19.6 (0.9–33.3)
Loop diuretics, n (%)		
Use at baseline	261 (80)	259 (79)
Patients with oral diuretic intensification during DB period	155 (48)	183 (56)
New loop diuretics initiated	22 (7)	37 (11)
Dose increase	133 (41)	146 (45)

Section C: What are the results?

7. Were the effects of intervention reported comprehensively?

☑ **Yes** ☐ No ☐ Can't tell

Power calculation?	With 654 patients, including 60% of patients in the monotherapy population, the power was approximately 80% for both primary endpoints in both the overall and monotherapy populations. (using a modified Andersen-Gill model with a robust variance estimator, with a 2-sided alpha = 0.05)
How were the **results expressed**?	• Binary outcomes (death, CV events): HR, 95% CI, p value • Continuous outcomes (6-MWT, KCCQ-OS score): Least-squares mean difference, 95% CI, p value
As mentioned previously: • What **outcomes** were measured, and were they clearly specified? • Were the **results** reported for **each outcome** in **each study group** at **each follow-up interval**? • Was there any **missing or incomplete data**? • Was there **differential drop-out** between the study groups that could affect the results? • Which **statistical tests** were used? • Were **p values** reported?	

Were potential sources of **bias** identified?

Section C: What are the results?

7. Were the effects of intervention reported comprehensively?

☑ **Yes** ☐ No ☐ Can't tell

Revised Cochrane risk-of-bias tool for randomized trials (RoB 2)

Domain	Risk of bias	Judgement
1	Randomization process	Low risk • Randomization used IRT with stratification (tafamidis use, ATTR type, NYHA/age) • Allocation concealment ensured
2	Deviations from the intended interventions (effect of assignment to intervention)	Low risk • Double-blind design; identical syringes • No evidence of protocol deviation
3	Missing outcome data	Some concerns • Some missing data due to death; handled via mixed effects model repeated measures (MMRM) and pattern-mixture model (PMM)
4	Measurement of the outcome	Low risk • Outcomes objectively defined or standardized • Clinical Events Committee (CEC) was blinded to treatment assignment
5	Selection of the reported result	Low risk • All primary/secondary outcomes pre-specified • Multiplicity (type I error rate) controlled via Hochberg procedure

Section C: What are the results?

8. Was the precision of the estimate of the intervention or treatment effect reported?

☑ **Yes** ☐ No ☐ Can't tell

- **95% confidence intervals (CIs)** reported for all key endpoints.

Section C: What are the results?

9. Do the benefits of the experimental intervention outweigh the harms and costs?

☐ Yes ☐ No ☑ **Can't tell**

- While benefits are clear, cost-effectiveness were not formally evaluated.

Benefits:

- Reduce risk of death from any cause and cardiovascular events.
- Preserve functional capacity and quality of life, particularly in patients with relatively early stages of the disease.

Harms:

- The incidence of adverse events among patients in the vutrisiran group was similar to or lower than that among the patients in the placebo group.

Table S6. Safety Summary in the Overall Population during the Double-Blind Exposure Period.

Event, n (%)	Vutrisiran (N = 326)	Placebo (N = 328)
At least 1 adverse event	322 (99)	323 (98)
Adverse events occurring in ≥15% of patients in either arm		
Cardiac failure	101 (31)	128 (39)
Covid-19	87 (27)	99 (30)
Atrial fibrillation	69 (21)	68 (21)
Gout	48 (15)	51 (16)
Dyspnea	43 (13)	51 (16)
Fall	42 (13)	69 (21)
Any serious adverse event*	201 (62)	220 (67)
Any severe adverse event*	158 (48)	194 (59)
Serious adverse events occurring in ≥5% of patients in either arm		
Cardiac failure	38 (12)	57 (17)
Atrial fibrillation	26 (8)	20 (6)
Cardiac failure acute	13 (4)	18 (5)
Cardiac adverse events	227 (70)	242 (74)
Cardiac serious adverse events	116 (36)	124 (38)
Any adverse event leading to treatment discontinuation	10 (3)	13 (4)
Any adverse event leading to death†	49 (15)	63 (19)

Section D: Will the results help locally?

10. Can the results be applied to your local population/in your context?

☑ **Yes** ☐ No ☐ Can't tell

- If patients have similar characteristics (e.g., wtATTR-CM, NYHA I–III), results are applicable.

[Taiwan] 359 cases, 114 deaths (Familial amyloidotic polyneuropathy)

Table 7. Common forms of transthyretin amyloid cardiomyopathy.

Hereditary ATTR-CM	Age at onset, y	Sex distribution	National/ethnic predominance	Cardic involvement	Other organ involvement
Val30Met (V30M) or pV50M	< 30 in early onset	Slight F > M	Portuguese, Swedish, and Japanese	Conduction disease more common than heart failure	Peripheral neuropathy
	> 60 in late onset				Autonomic neuropathy
Val122Ile (V122I) or pV142I	60-65 (older age at onset in women)	Slight M > F	Afro-American	Common	Peripheral neuropathy likely
			Afro-Caribbean		Bilateral carpal tunnel syndrome
Thr60Ala (T60A) or pT80A	> 60	Unknown	Irish	Common	Autonomic and peripheral neuropathy
Ala97Ser	> 50	Slight M > F	Taiwanese	Common	Sensory-motor
					Autonomic and peripheral neuropathy

Wild ATTR-CM	Age at onset, y	Sex distribution	National/ethnic predominance	Cardic involvement	Other organ involvement
TTRwt	70-75	80%-90% male	None	Common	Bilateral carpal tunnel syndrome, spinal stenosis, biceps tendon rupture

*The overall prevalence may be underestimated due to underdiagnosis.

Acta Cardiol Sin. 2023;39(4):511-543.

衛生福利部國民健康署. 114 年 5 月罕見疾病通報個案統計表. (統計期間：個案數至114年5月31日及死亡數至113年12月31日)

Section D：Will the results help locally?

11. Would the experimental intervention provide greater value to the people in your care than any of the existing interventions?

☐ Yes ☐ No ☑ **Can't tell**

藥品名稱	成分及含量	健保初核價格	給付規定

Vyndamax	Tafamidis
	61mg
		7,240	**wtATTR&hATTR-CM**，每日限使用 1 粒，限給付 45 個月(113/2/1)
			• 符合：
			  • 排除：eGFR<25、AL amyloidosis
			  • PYP scintigraphy scan visual score=Grade 3 + cardiac biopsy
			  • NYHA class 2&3
			  • NT-proBNP ≥ 600 pg/ml or BNP ≥ 100 pg/ml
			  • Interventricular septal wall thickness >12mm and Left ventricular posterior wall dimensions >12mm
			  • LVEF≥40%
			• 停用：接受心室輔助系統、心臟移植或肝臟移植治療、NYHA達class 4

Amvuttra	Vutrisiran 50 mg/mL, 0.5 mL	1,718,403	**Transthyretin familial amyloidotic polyneuropathy (TTR-FAP)** (114/5/1) • 符合： • polyneuropathy disability [PND] stage Ⅱ, Ⅲa~Ⅲb or familial amyloidotic polyneuropathy [FAP] stage Ⅰ, Ⅱ **尚未核可用於ATTR-CM**

Take home message

	**Vutrisiran** (**Amvuttra**, Alnylam)
Regulatory approval	• hATTR-PN (☑ Taiwan) • wtATTR & hATTR-CM
Class & Formulation	• GalNAc-conjugated siRNA • Injection
Mechanism of action (MOA)	Binds proteins that form the RISC, with release of passenger strand and antisense strand bound to RISC binds to target TTR mRNA, which is then cleaved by Argonaute2.
Dose	**25 mg, SC, Q3M**
Additional medication	vitamin A supplementation daily
In HELIOS-B trial: **Among patients with ATTR-CM, treatment with vutrisiran led to a lower risk of death from any cause and cardiovascular events than placebo and preserved functional capacity and quality of life.**	

- hATTR-PN: hereditary transthyretin-mediated amyloidosis polyneuropathy
- GalNAc: N-acetylgalactosamine

About small nucleic acid therapeutics

Signal Transduction and Targeted Therapy

www.nature.com/sigtrans

REVIEW ARTICLE OPEN

Landscape of small nucleic acid therapeutics: moving from the bench to the clinic as next-generation medicines

Mohan Liu[1], Yusi Wang[1], Yibing Zhang[1], Die Hu[1], Lin Tang[1], Bailing Zhou[1] and Li Yang[1]✉

The ability of small nucleic acids to modulate gene expression via a range of processes has been widely explored. Compared with conventional treatments, small nucleic acid therapeutics have the potential to achieve long-lasting or even curative effects via gene editing. As a result of recent technological advances, efficient small nucleic acid delivery for therapeutic and biomedical applications has been achieved, accelerating their clinical translation. Here, we review the increasing number of small nucleic acid therapeutic classes and the most common chemical modifications and delivery platforms. We also discuss the key advances in the design, development and therapeutic application of each delivery platform. Furthermore, this review presents comprehensive profiles of currently approved small nucleic acid drugs, including 11 antisense oligonucleotides (ASOs), 2 aptamers and 6 siRNA drugs, summarizing their modifications, disease-specific mechanisms of action and delivery strategies. Other candidates whose clinical trial status has been recorded and updated are also discussed. We also consider strategic issues such as important safety considerations, novel vectors and hurdles for translating academic breakthroughs to the clinic. Small nucleic acid therapeutics have produced favorable results in clinical trials and have the potential to address previously "undruggable" targets, suggesting that they could be useful for guiding the development of additional clinical candidates.

Signal Transduction and Targeted Therapy (2025)10:73 ; https://doi.org/10.1038/s41392-024-02112-8

Thank you

Reference

- Ruberg FL, Maurer MS. Cardiac Amyloidosis Due to Transthyretin Protein: A Review. *JAMA*. 2024;331(9):778-791.
- Marianna Fontana. Cardiac amyloidosis: Epidemiology, clinical manifestations, and diagnosis. In: UpToDate, Connor RF (Ed), Wolters Kluwer. (Accessed on May 11, 2025.)
- Shetty M. A cardiology fellow's take-home points from ASNC's Webinar: Cases in Tc 99m-PYP evaluation of ATTR cardiac amyloidosis - interpretation and reporting. *J Nucl Cardiol.* 2021;28(2):604-609.
- Marianna Fontana. Cardiac amyloidosis: Treatment and prognosis. In: UpToDate, Connor RF (Ed), Wolters Kluwer. (Accessed on May 15, 2025.)
- Aimo A, Castiglione V, Rapezzi C, et al. RNA-targeting and gene editing therapies for transthyretin amyloidosis. *Nat Rev Cardiol.* 2022;19(10):655-667.
- Androsavich JR. Frameworks for transformational breakthroughs in RNA-based medicines. *Nat Rev Drug Discov.* 2024;23(6):421-444.
- Keam SJ. Vutrisiran: First Approval. *Drugs.* 2022;82(13):1419-1425.
- Spertus JA, Jones PG, Sandhu AT, Arnold SV. Interpreting the Kansas City Cardiomyopathy Questionnaire in Clinical Trials and Clinical Care: JACC State-of-the-Art Review. *J Am Coll Cardiol.* 2020;76(20):2379-2390.
- Wang CC, Chang WT, Lin YH, et al. 2023 Expert Consensus of the Taiwan Society of Cardiology on the Diagnosis and Treatment of Cardiac Amyloidosis. *Acta Cardiol Sin.* 2023;39(4):511-543.