Relationship of binding-site occupancy, transthyretin stabilisation, and disease modification in tafamidis treated transthyretin amyloid cardiomyopathy patients

Tess, D. A., Maurer, T. S., Li, Z., Bulawa, C., Fleming, J., & Moody, A. T. (2022). Relationship of binding-site occupancy, transthyretin stabilization and disease modification in patients with tafamidis-treated transthyretin amyloid cardiomyopathy. *Amyloid*, 1-12.

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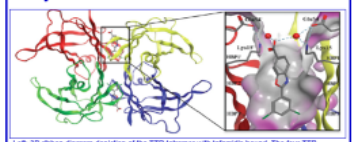
Tafamidis is indicated for the treatment of wild-type and hereditary ATTR-CM in adults to reduce cardiovascular mortality and cardiovascular-related hospitalization[4]

The Phase 3 Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT) demonstrated the efficacy and safety of Tafamidis for patients with ATTR-CM[1]

- Tafamidis binds in one or both of the two thyroxine binding pockets at the TTR dimer-dimer interface
  - **Tafamidis inhibits tetramer dissociation, resulting in stabilization and increased abundance of the tetramer[2]**

Crystal structure of tafamidis bound to TTR



Left: 3D ribbon diagram depiction of the TTR tetramer with tafamidis bound. The four TTR monomers are individually colored. Right: Magnified image of tafamidis bound in one of the thyroxine (T4) binding sites.

Image reproduced from Bulawa CE, et al. *Proc Natl Acad Sci USA* 2012[3]

*The FDA approved dosing is either tafamidis meglumine 80mg (four 20 mg tafamidis meglumine capsules) orally once daily OR tafamidis free acid 61mg (one 61mg tafamidis free acid capsule) orally once daily.

ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; QD, every day; TTR, transthyretin.
1. Maurer MS, et al. N Engl J Med 2018;379(11):1007–16. 2. Maurer MS, et al. Circ Heart Fail 2015;8(2):519–26. 3. Bulawa CE, et al. Proc Natl Acad Sci USA 2012;109(24):9629–34. 4. VYNDAQEL® (VYNDAMAX®) Prescribing Information. New York, NY: Pfizer Inc.5. Nativi-Nicolau J, et al. Curr Opin Cardiol 2016;31:271–6.

Study Design

In this study, TTR stabilization was defined as the degree to which the tetramer dissociation rate decreased in human plasma following the 20 mg and the approved 80 mg once daily (QD) doses of tafamidis meglumine.[1]

This definition was chosen because the change in TTR dissociation rate under therapeutic and physiologic conditions is the most clinically relevant measure of pharmacologic activity for this class.[2]

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Data obtained from ITC, subunit fraction exchange, and albumin binding experiments were simultaneously modelled to estimate a common set of **tafamidis meglumine binding dissociation constants to TTR ($K_{d,1}$ and $K_{d,2}$) and albumin ($K_{d,A}$)**

Clinical data from the Phase 3 ATTR-ACT study was used in this analysis[1]

TTR concentrations over time were simultaneously characterized as a function of inter-individual variability in TTR production rate, and also as a function of tafamidis meglumine concentration, to develop **population TTR binding and concentration–time models**

Population disease progression response–time models were developed using three disease progression measures:

- N-terminal pro-B natriuretic peptide (NT-proBNP) plasma levels
- Kansas City Cardiomyopathy Questionnaire Overall Score (KCCQ-OS) score
- 6-minute walk test (6MWT) distance

6MWT, 6-minute walk test; ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; KCCQ-OS, Kansas City Cardiomyopathy Questionnaire Overall Score; NT-proBNP, N-terminal pro-B-type natriuretic peptide; QD, once daily; TTR, transthyretin.
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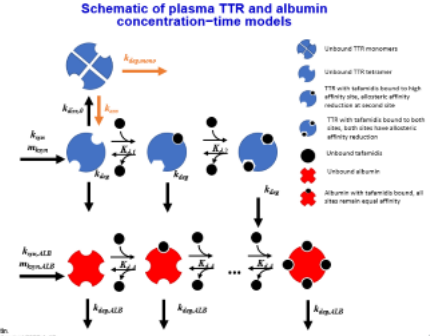
The final model structure used to characterize the relationship between tafamidis meglumine and TTR concentrations is shown in the figure

Schematic of plasma TTR and albumin concentration–time models



Use of these values provided **accurate predictions** of the concentration-dependent unbound fraction of tafamidis meglumine in human plasma and confidence in the TTR occupancies inferred by these parameters

TTR, transthyretin.
Tess DA, et al. *Amyloid* 2022;1-12.

Results: Relationship between TTR occupancy, TTR stabilization, and clinical measures related to disease progression

Tafamidis meglumine fractional TTR occupancy

- Population average plasma tafamidis meglumine concentrations associated with 20 and 80 mg QD doses were 8.8 and 35 µM, respectively
- As such, tafamidis meglumine doses are estimated to achieve population average site occupancy as follows:

TTR occupancy	Single site	Dual site
20 mg	73%	1%
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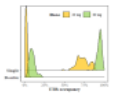

- Modelling, consistent with in vitro results and literature reports, found that the dissociation rate of TTR in the presence of singly bound tafamidis was negligible
- With a TTR single site occupancy of 94%, this results in tafamidis 80 mg achieving TTR stabilisation that is close to the estimated maximum stabilisation that would be achieved with 100% TTR occupancy

NT-proBNP concentration-time model

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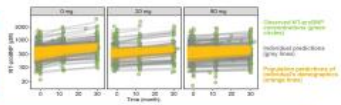
NTproBNP concentrations over time had a linear relationship with unbound TTR concentration



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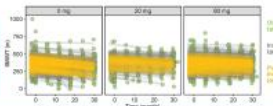

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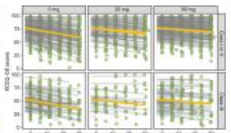

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Conclusions

These results indicate that **singly bound tafamidis** provides **near-complete TTR stabilization**

The model demonstrates that the clinically approved dose of **80 mg tafamidis meglumine achieves a near- maximum (>90%) effect** on both TTR stabilization and disease-relevant measures

These findings support the value of TTR stabilization as a **clinically effective** treatment for ATTR-CM

ATTR-CM, transthyretin amyloid cardiomyopathy; TTR, transthyretin.
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Pfizer Rare Diseases

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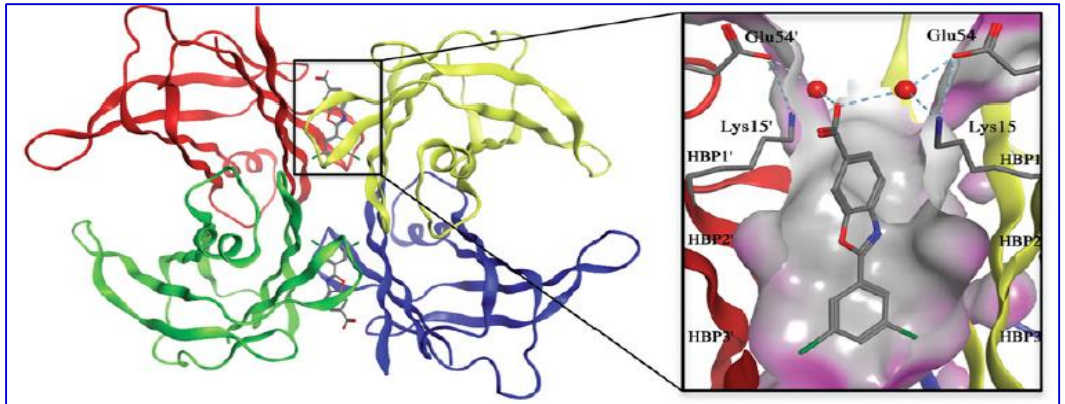

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Transthyretin Cardiomyopathy (ATTR-CM) with Tafamidis Meglumine in the ATTR-ACT study

Current median survival expectations for untreated patients with ATTR-CM are **~2 to 5 years** from diagnosis.[1–3] During this time, patients experience **declining health and quality of life** (QoL).[1,3]

ATTR-ACT was a Phase 3, international, randomized, double-blind, placebo-controlled evaluation of the safety and efficacy of tafamidis meglumine in patients with ATTR-CM.[4]

The primary outcomes showed that tafamidis meglumine **significantly reduced mortality and cardiovascular (CV)-related hospitalizations** compared with placebo over 30 months.[4]

Tafamidis was well tolerated, with a safety profile comparable to placebo.[4]

Patients treated with tafamidis demonstrated better overall scores in 6MWT distance, NT-proBNP concentration, KCCQ-OS, and PGA of overall health score compared to placebo despite both tafamidis and placebo treated patients showing progressively declining health at Month 30.[4–7]

ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; ATTR-CM, transthyretin amyloid cardiomyopathy; CV, cardiovascular; KCCQ-OS, Kansas City Cardiomyopathy Questionnaire Overall Summary; NT-proBNP, N-terminal pro-brain natriuretic peptide; QoL, quality of life.; 6MWT, 6-minute walk test

1. Nativi-Nicolau J, et al. *ESC Heart Fail* 2021;8(5):3875–84; 2. Grogan M, et al. *J Card Fail* 2017;23(8):S41; 3. Lane T, et al. *Circulation* 2019;140(1):16–26; 4. Maurer MS, et al. *N Engl J Med* 2018;379(11):1007–16; 5. Damy T, et al. *Eur J Heart Fail* 2021;23(2):277–85; 6. Hanna M, et al. *Am J Cardiol* 2021;141:98–105; 7. Hanna M, et al. *JACC Adv.* 2022 Dec, 1 (5) 1–8.

TTR Stabilization in patients with Tafamidis treated ATTR-CM

TTR stabilization has multiple meanings as per the current literature and is often associated with methods that do not measure the tetramer dissociation rate and/or include non-physiological conditions such as addition of denaturants

The receptor occupancy study by Tess et al leverages physiologically relevant measures of tafamidis binding to TTR tetramer and albumin in conjunction with therapeutically relevant patient data from ATTR-ACT to establish the relationship **between TTR occupancy, TTR stabilization, and clinical measures related to disease progression** in patients with ATTR-CM treated with tafamidis meglumine

The model-based characterization of these data is used to **assess the degree to which the clinically approved daily 80 mg dose of tafamidis meglumine achieves the full potential** for TTR kinetic stabilizers

ATTR-ACT, Tafamidis in Transthyretin Cardiomyopathy Clinical Trial; TTR, transthyretin.
Tess DA, et al. *Amyloid* 2022 Nov, 1-12.

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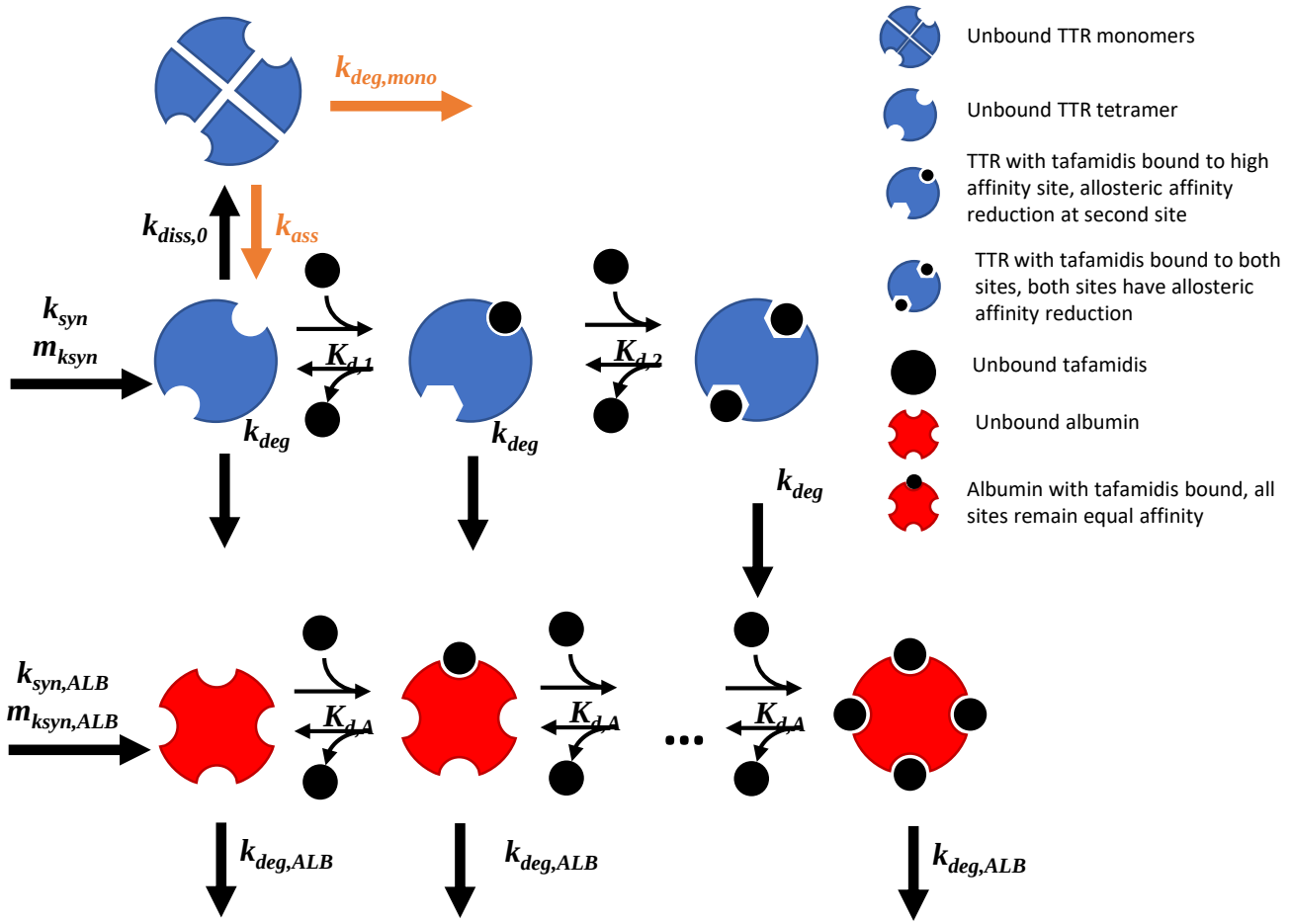

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Results: Characterization of tafamidis meglumine binding to TTR and albumin in plasma

- The two known tafamidis binding partners in human plasma are TTR and albumin
- In order to create a dynamic model of how TTR and tafamidis concentrations relate to TTR occupancy by tafamidis and unbound concentrations of TTR in human plasma:
  - The binding affinity estimates of tafamidis to TTR and albumin were calculated
  - Creation of population albumin and TTR concentration time models accounted for albumin and TTR concentrations changes over time

Binding affinity estimates

Modeling data from ITC, subunit fraction exchange, and equilibrium dialysis provided a reasonable characterization and precise binding affinity estimates:

- $K_{d,1}$ estimates of 5.08 nM (4.1-6.3)
- $K_{d,2}$ estimates of 203 nM (160-250)
- $K_{d,A}$ value of 3.53 µM (3.0-4.2)

(95% confidence interval)

Population albumin and tafamidis concentration–time models

- The models used to estimate change in albumin or tafamidis concentrations used the rates in the table below
- The structure of these time-dependent aspects in the final model were determined based on the ability to provide a good fit to the data

k_{syn}	TTR production rate
mk_{syn}	Linear slope of change of k_{syn}
$k_{deg,\ 0}$	Unbound TTR degradation rate
$k_{deg,\ mono}$	TTR monomer degradation rate
$k_{diss,\ 1}$	Single-bound tafamidis-bound TTR dissociation rates to monomer
$k_{diss,\ 2}$	Double-bound tafamidis-bound TTR dissociation rates to monomer
k_{ass}	Association rate of monomer back to tetramer
$k_{syn,\ ALB}$	Albumin production rate
$mk_{syn,\ ALB}$	Linear slope of change of $k_{syn,\ ALB}$
$k_{deg,\ ALB}$	Albumin fixed degradation rate

ITC, isothermal titration calorimetry; TTR, transthyretin.

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Tafamidis meglumine fractional TTR occupancy

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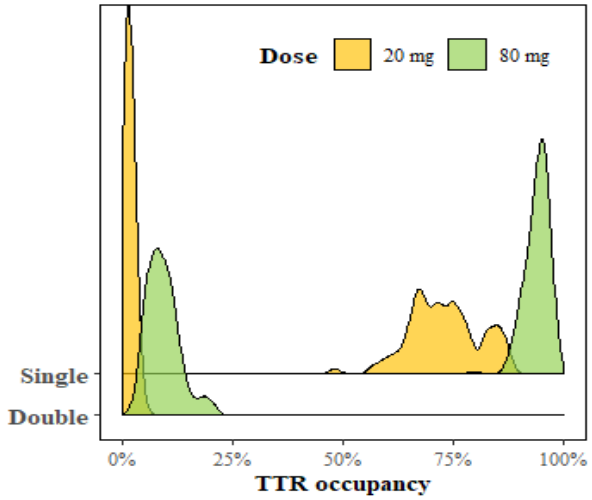

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TTR, transthyretin; QD, once daily.
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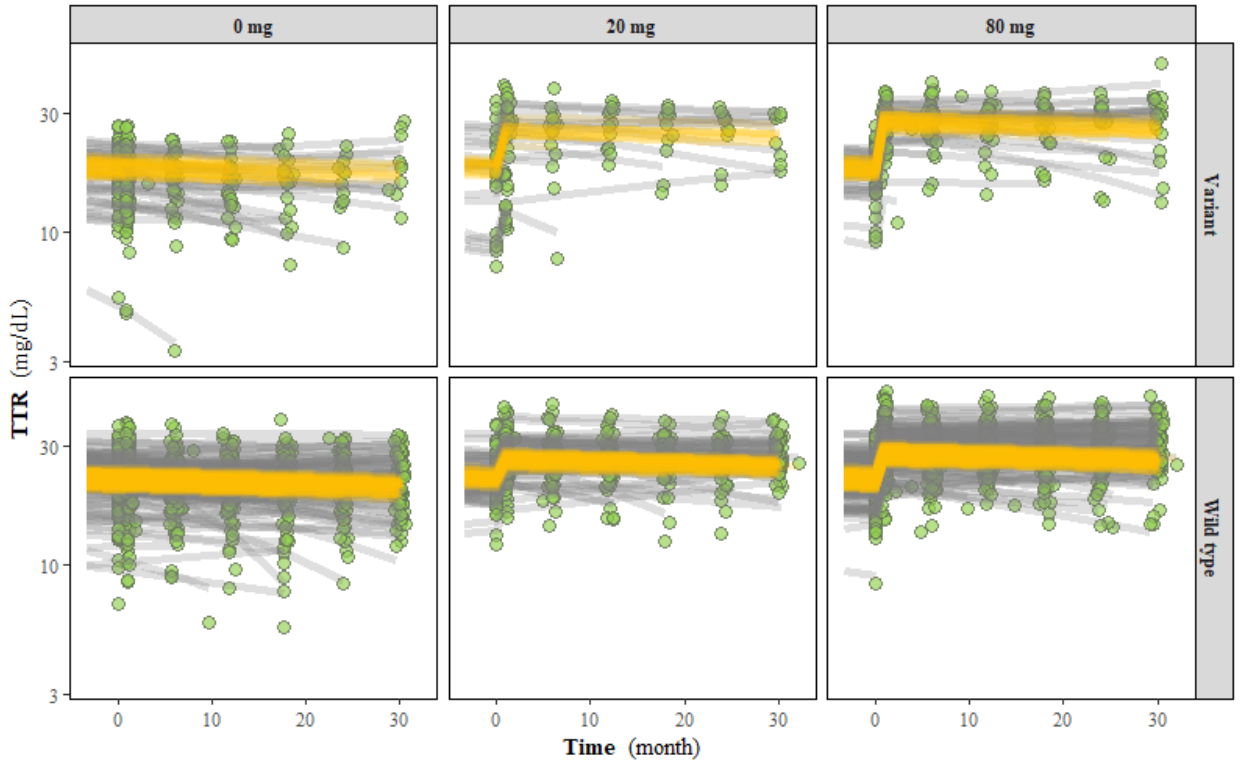
Population TTR binding and concentration–time model

- The model captured key characteristics of **placebo patients**:
  - **Lower initial TTR concentration in variant vs wild-type** captured by two-fold faster tetramer dissociation rate
  - **Decrease in TTR concentration over time**
- The **effect of Tafamidis on TTR concentration** was captured by a **decrease in the tetramer dissociation rate with a linear dependence on TTR occupancy**



Observed TTR concentrations (green circles)

Individual predictions (grey lines)

Population predictions of individual's demographics (orange lines)

TTR, transthyretin.
Tess DA, et al. *Amyloid* 2022;1–12.

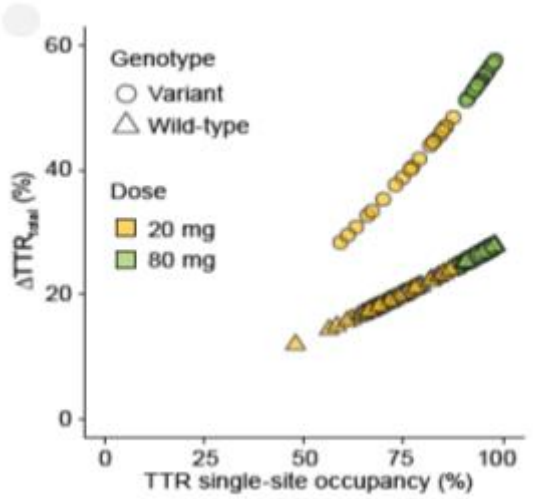
Effect of tafamidis meglumine on total and unbound TTR concentrations

- **Tafamidis meglumine 20 and 80 mg increased total TTR concentrations** by a population average of 19% and 26% in wild-type TTR genotype patients, and by 40% and 55% in variant TTR genotype patients, respectively

This is near what would be expected for **complete stabilization** of TTR (29% [wild-type] and 59% [variant TTR genotype]), which indicates that the approved dose of tafamidis meglumine provides a **degree of stabilization that is near the maximum achievable** (i.e. TTR dissociation <10%)

- **Unbound TTR** decreased by an average of 67% and 92% with tafamidis meglumine doses of 20 and 80 mg, respectively

Total TTR



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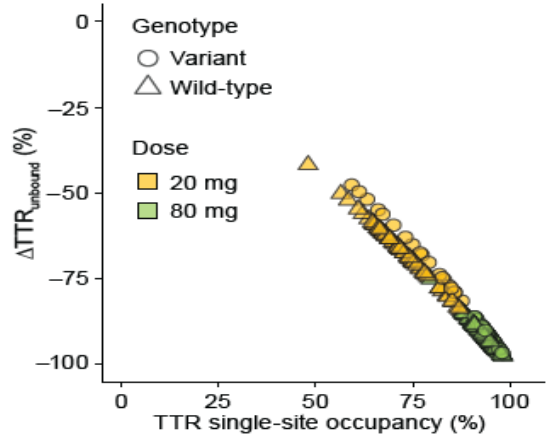

TTR, transthyretin; ΔTTR, estimated fractional change from baseline in TTR concentration.
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NT-proBNP concentration-time model

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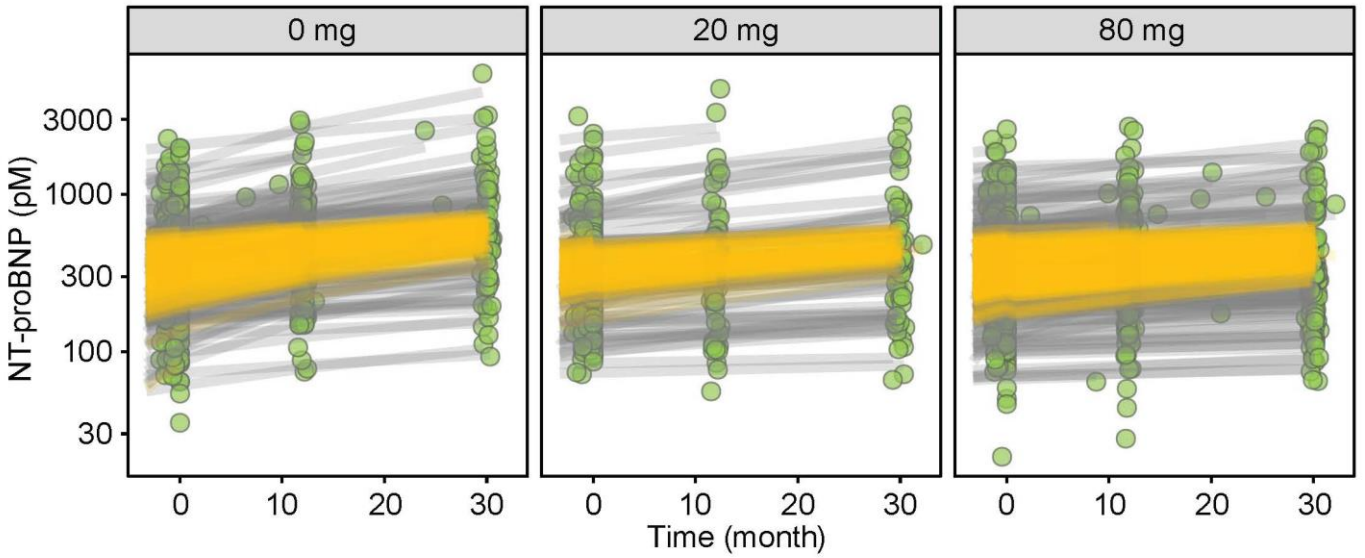

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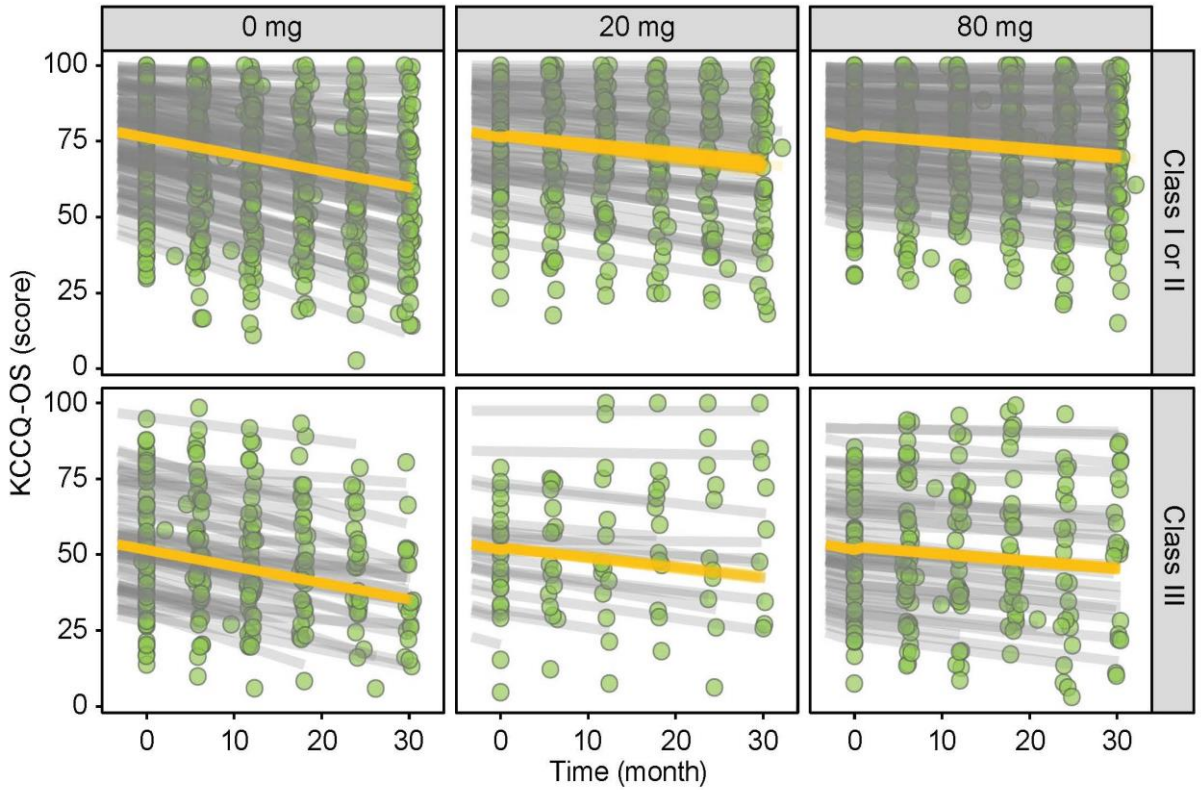

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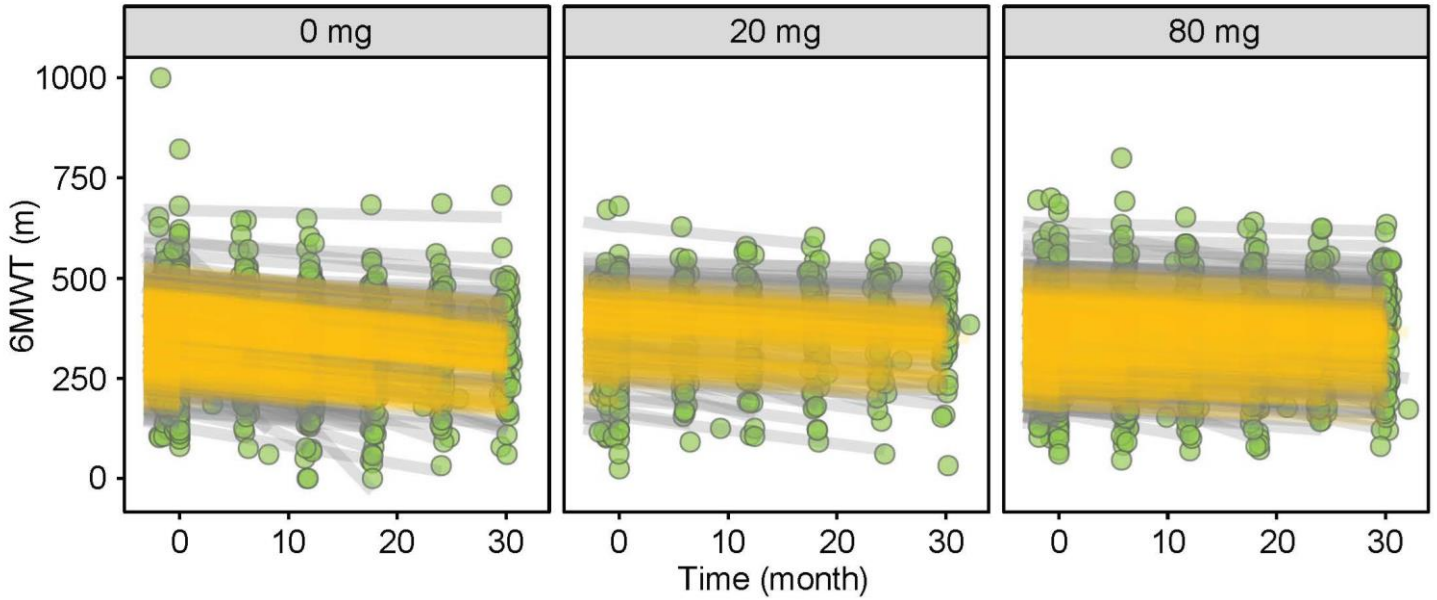

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