

A new therapy for transthyretin amyloidosis, no longer an orphan condition

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Amyloid cardiomyopathy is a condition characterized by intra-myocardial deposit of protein-like material, in fibrillar shape (amyloid), which presence determine a progressive thickening and stiffening of the cardiac walls leading to a cardiac dysfunction. The proteins most often involved with cardiac amyloid are the light chains of the immunoglobulin, typical of amyloidosis AL, and transthyretin, responsible for transthyretin amyloidosis, in both its forms, hereditary and wild type. An accurate estimate of the incidence of cardiac amyloidosis is still difficult due to the variety and complexity of the clinical presentation of the condition. Nonetheless, the condition has stimulated the interest of the scientific community, so that a specific diagnostic path has been developed, beginning from the clinical suspicion and first-line testing, such as electrocardiogram, echocardiogram, and blood work, to progress to the diagnostic confirmation using more sophisticated testing such as magnetic resonance, scintiscan, and eventually cardiac biopsy. To understand and recognize this condition is very important, stemming from the availability of ‘aetiology oriented therapies’ (designed to prevent, control and possibly regress amyloid deposition), which should be added to the ‘supportive therapies’, used for the treatment of the complication of the condition, namely heart failure.

What is amyloidosis?

The term amyloid describes the deposition in the extracellular space of normal or abnormal proteins circulating in the blood, in a highly characteristic insoluble and fibrillar form, derived from a disorder in the process of folding of the amino acid chain that leads them to aggregate and precipitate at tissue level in the form of rigid and linear fibrils.¹

There are about 30 proteins that, in humans, can form amyloid deposits, virtually in any part of the body.² The ‘amyloidosis’ represent the clinical syndromes resulting from these deposits and are classified as localized or systemic, acquired or inherited: it is important to have a good

case-by-case classification that leads to a precise aetiological diagnosis, given the variability of natural history and of treatments between the various clinical forms, ranging from asymptomatic deposits, to localized diseases, to rapidly fatal systemic forms that can affect several vital organs simultaneously.^{1,2}

The heart represents one of the target organs in which amyloid is most frequently deposited, giving rise to the so-called ‘amyloidotic cardiomyopathy’ (AC). In this condition, the more the deposits progress, the more the walls of the heart become thick and rigid and the contractile function worsens. Apart from myocardial tissue, infiltration can also involve the valve apparatus and the conduction system.

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How does cardiac amyloidosis occur?

The AC is still a diagnostic challenge for the clinician, as, besides being a rare disease, it manifests itself in an extremely heterogeneous way regardless of the aetiological form, with consequent delay or even lack of diagnosis.

Regardless of the specific aetiology, and therefore of the particular precursor protein, the natural history of the AC is marked by a progressive thickening of the cardiac walls, which determines over time a compromise of the contractile function, which results in water retention and finally the onset of congestive heart failure. Alongside this dimension of chronically infiltrative myocardial disease, an acute 'toxic' component has been documented linked to the effect that some amyloidogenic proteins can exert directly on the myocardium. This direct toxic action is responsible, for example, for the extraordinarily high production of NT-proBNP that accompanies the amyloidotic cardiomyopathies as well as stable and chronic troponins release.^{3,4}

The most common symptoms of AC include dyspnoea, with progressive reduction of exercise tolerance, formation of dependent oedema, and the sensation of abdominal bloating. The patient, in the full-blown form, presents mainly pictures of right or bi-ventricular heart failure.

Syncope presentations are also not infrequent, in which the patient reports syncope and pre-syncope episodes or palpitations, which may imply the presence of brady or supraventricular or ventricular tachyarrhythmias but also an expression of severe orthostatic hypotension.

Sometimes the patient may also complain of stress angina which may be secondary to the presence of amyloid deposits at the level of the coronary microcirculation.^{3,4}

Although the initial presentation may be exclusively cardiac, with the above-mentioned manifestations, we must, however, not overlook some signs and symptoms, secondary to the involvement of other organs, whose presence and intensity vary according to the specific aetiology, including: history of carpal tunnel, peripheral neuropathy (tingling and numbness of hands and feet), or autonomic (urinary incontinence and impotence), soft tissue infiltration (macroglossia), vertigo (caused by orthostatic hypotension), foamy urine (caused by protein loss in urine), symptoms of gastrointestinal nature (vomiting, diarrhoea, constipation), unexplained bleeding or bruising (particularly around the eyes and arms), and weight loss (often).

What are the characteristics of transthyretin-amyloidosis (ATTR)?

Transthyretin (TTR) is a transport protein that is synthesized mainly (~99%) by the liver and that transports thyroxine (tT4) and retinol-binding protein at the blood level. From its function derives the name 'transthyretin': TRANS (ports) THY (roxin) and RETIN (ol). The structure of the TTR is tetrameric, i.e. formed by four identical subunits (monomers).

There are two types of amyloidosis derived from TTR deposition: a genetic form known as 'hereditary TTR amyloidosis' (ATTRm) and a non-hereditary form known as 'wild

type' TTR amyloidosis (ATTRwt, also known as 'senile systemic amyloidosis').^{5,6}

In the first case, the disease occurs in the presence of a defect in the gene that codes for the amino acid chain of the protein, a defect generally inherited from only one of the two parents who has a 50% chance of transmitting it to each child (autosomal dominant transmission, for most heterozygous, homozygous cases are anecdotal). The presence of a mutation gives the protein a more unstable conformation, the tetramer is more susceptible to dissociation into monomers, an essential step for starting the amyloidogenic process. However, even if the mutation is present from birth, and therefore an abnormal protein is produced throughout life, it continues to maintain its conformation until adulthood, when the protein begins to precipitate in fibrillar form and to form deposits. All this suggests the existence of further pathophysiological mechanisms in addition to the intrinsic instability of the mutated protein.

About a hundred possible TTR mutations are known to date and the clinical presentation of the disease (phenotype) depends on the particular underlying genetic anomaly (genotype): there is a vast heterogeneity of the clinical spectrum ranging from an exclusively neurological involvement (peripheral or autonomic neuropathy), to cases with exclusively cardiac involvement (infiltrative cardiomyopathy), passing through situations in which the two presentations coexist in a mixed clinical picture.⁷ Apart from the specific TTR mutation, other determinants of the known phenotypic variability are the geographical area to which patients belong, the type of aggregation (endemic or non-endemic), the type of conformation assumed by the amyloid fibrils in the infiltrated tissues, the sex of the patient and parent who transmits the anomaly and other environmental factors yet to be identified. It follows that the presence of the mutation in itself does not lead to the development of the disease (incomplete penetrance) and inter and intra-familial expressiveness can in turn be very variable. The cardiologist must be aware of the existence of those TTR mutations that are expressed through cardiac symptoms (about 17% of cases),⁷ including, of particular relevance in Italy, the Ile68Leu mutation: the latter, probably endemic in the regions of Emilia-Romagna and Tuscany,⁷ represents the cause of 74% of ATTRm amyloidosis cases with exclusively cardiac phenotype recorded in our country.⁷

The ATTRwt instead derives from the deposition of TTR not mutated (i.e. 'wild type') which, despite the absence of mutations, is intrinsically amyloidogenic. Although the deposits are diffusely localized throughout the body (in fact systemic deposits are found at the autopsy examination of 25% of individuals over the age of 80), the disease manifests itself clinically, mostly at cardiac level, particularly in male subjects over 65 years.⁷⁻¹⁰

Since the heart is the mainly involved site, the disease presents with varying degrees of heart failure. It is very interesting to note that the only other two manifestations of this condition are represented by the history of carpal tunnel syndrome (compression of the median nerve at the wrist level) and/or cauda equina syndrome (nerve compression at the lumbar level), which generally occur some years before cardiac symptoms.⁹

Both forms of TTR amyloidosis lead to a progressively slower disease than that of AL amyloidosis and most affected patients survive for several years after the onset of the first signs and symptoms. The incidence of sudden death in these forms is decidedly less frequent but always mainly caused by electromechanical dissociation. Disease course and survival after diagnosis depend on some factors common to the two conditions (degree of heart failure, age of the patient, comorbidity); in the cases of ATTRm, the specific TTR mutation plays a role in the natural history of the disease, resulting in the most aggressive and debilitating early-onset neuropathic forms compared to late forms with predominantly cardiac manifestations.

How do you get the diagnosis of cardiac amyloidosis?

Being a rare condition characterized by somewhat non-specific symptoms, the disease is still diagnosed with some delay. The clinical suspicion remains the basis on which to construct the most appropriate diagnostic procedure.

From suspicion . . .

Electrocardiogram (ECG) and echocardiogram are the first level instrumental tests for the diagnosis of AC.^{10,11} In a patient with cardiac symptoms, the presence of a thickening of the cardiac walls at the echocardiographic examination, in the absence of plausible causes of left ventricular hypertrophy (hypertensive cardiomyopathy, etc.) should lead to suspect not only the most known and diagnosed hypertrophic and hypertensive cardiomyopathy but also an infiltrative disease such as amyloidosis. In this context the integrated ECG/echocardiogram reading provides useful information for the diagnostic suspicion, in particular when the presence of left ventricular hypertrophy at the echocardiogram correspond to some electrocardiographic alterations, including low voltages peripheral, pseudonecrosis patterns, conduction delays, repolarization alterations.¹¹ In particular, the presence of low voltages at the ECG is considered pathognomonic of AC, but it is nevertheless important to remember that the low ECG voltages are frequent (50-60%) only in AL amyloidosis, but less common in TTR forms (20-30%), stressing the importance of not excluding a diagnosis of AC even in the absence of this ECG pattern.

The most relevant echocardiographic features in the AC include¹¹: symmetrical thickening of the walls of the left ventricle in the absence of ventricular cavity dilation, thickening of the valvular leaflets, inter-atrial septum and free wall of the right ventricle, bi-atrial dilation, pericardial effusion. From a functional point of view, the ejection fraction is more often 'preserved', but the longitudinal systolic function can be altered already in the early stages of the infiltrative process, even in the absence of a significant increase in wall thicknesses. The most recent methods for the analysis of myocardial deformability, in particular of the longitudinal strain (speckle tracking) have allowed us to identify a more pronounced pattern of left ventricular dysfunction at the base of the ventricle than at the apex.

This finding is highly useful for the differential diagnosis between AC and other forms of left ventricular hypertrophy (22). Diastolic dysfunction is a frequent finding, but a real restrictive pattern is highlighted only in the advanced stages of the disease.

Since AL amyloidosis is by far the most frequent cause of AC, blood and urine tests are essential to rule out the presence of a plasma cell dyscrasia: electrophoresis and immune-fixation, serum and urinary, associated with serum levels of free light chains, in fact allow to detect the presence of a monoclonal protein in 98-99% of cases. These tests are important both for the diagnosis and for the evaluation of treatment response and subsequent monitoring.

To determine both the degree of cardiac involvement and the degree of myocardial damage, the blood values of NT-proBNP and of Troponin are measured, useful above all in cases without significant renal dysfunction.¹¹ To date, there is no laboratory test capable of providing 'per se' diagnosis of ATTR.

. . . to confirmation

Once the AC is suspected, it is necessary to reach a definitive diagnosis. The endomyocardial biopsy still represents, in general, the gold standard in the diagnosis of AC, given the extreme accuracy both in confirming the presence of amyloid (apple-green birefringence after staining with Congo-red under a polarized light microscope) and in characterizing the protein contained in deposits (immunohistochemistry and/or proteomics analysis).³⁻⁵

At present, however, the use of endomyocardial biopsy has been reduced by the increasing diagnostic accuracy offered by other non-invasive imaging methods and in particular by scintigraphy with bone tracers.¹²

Cardiac magnetic resonance imaging is a particularly important non-invasive instrumental investigation for the diagnosis of AC,¹³ mainly thanks to the use of gadolinium, a tracer that accumulates at the extracellular level with a non-coronary pathognomonic subendocardial distribution (late gadolinium enhancement). Recently the 'T1 mapping' technique was introduced, in which the quantitative signal coming from myocardium was measured first (native T1) and after the administration of gadolinium, a signal that resulted increased in both forms of Combined AC; the T1 mapping and measurement of extracellular volume (ECV) after gadolinium administration can describe three important aspects: the amount of amyloid and the degree of infiltration through the measurement of ECV, the amount of oedema through native T1 and the cellular response through measurement of intracellular volume. This instrumental investigation can therefore be of great help both in the understanding of the pathophysiological processes that determine the onset of the disease and in the monitoring of its progression and response to therapy.^{11,13}

Although magnetic resonance imaging offers a diagnostic accuracy superior to the echocardiogram for the recognition of AC, this too is not able to distinguish between the different aetiological forms. Nuclear medicine is more accurate in the differential diagnosis. In particular, bone scintigraphy with ^{99m}Tc-DPD allows to identify the cases of AC

from TTR, in which the radioisotope is avidly picked up by the myocardium with a pattern that has not yet been found in any other type of non-AC. This counteracting uptake is absent or very mild in the AL forms and in other extremely rare forms of AC. Furthermore, the method is able to detect TTR deposits at a pre-clinical stage of the disease, when echocardiography, biomarkers and sometimes magnetic resonance are still normal. In the suspicion of an AL form, aspirate and bone biopsy are also performed to characterize the plasmacell clone.¹¹⁻¹⁴ Finally, in the suspicion of a TTR form, it is important to search for mutations in the TTR gene which, if negative, directs the diagnosis towards the ATTRwt.

Figure 1 gives a summary of how AC can be presented in various diagnostic investigations.

It is important to emphasize that, especially in elderly patients, two situations may overlap which could constitute a source of error in the final diagnosis. On the one hand, in fact, there can be a simultaneous presence of TTR

amyloidosis and a monoclonal protein detectable in serum and/or urine secondary to a monoclonal gammopathy of uncertain significance (MGUS), the latter having a 5% incidence in the population aged >65 years.¹⁴ It follows that the mere presence of the monoclonal protein does not allow to label with certainty the amyloidosis as AL, and instead it is important to proceed with further diagnostic investigations, including the endomyocardial biopsy, in order to typify the amyloid substance and dispel any doubts about the nature of cardiac deposits. Alternatively, typing of the amyloid substance can be performed on biopsy of peri-umbilical fat.

What therapy can be offered to the patient with ATTR?

In the presence of AC the therapeutic approach is two-fold: treatment of complications ('supportive therapy'),

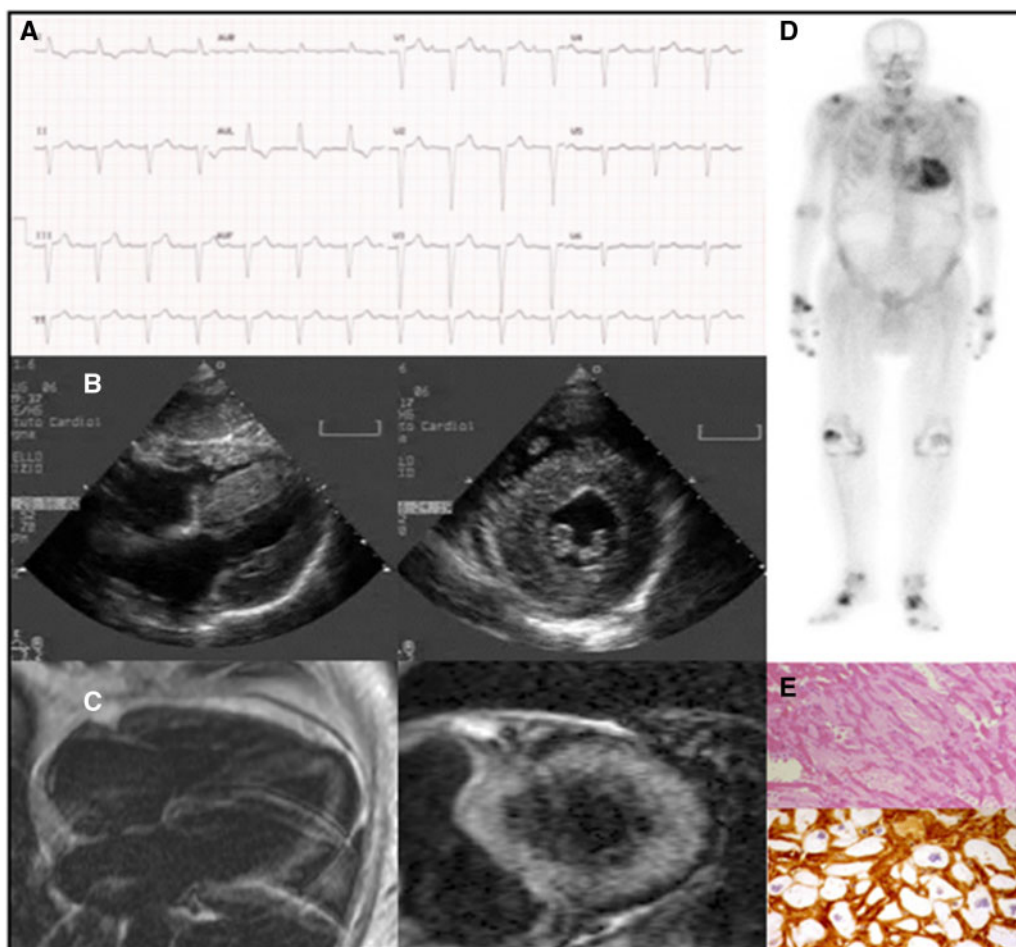


Figure 1 Principal diagnostic tests in a patient with cardiac amyloidosis. (A) Electrocardiogram: presence of preserved QRS voltages, first degree atrio-ventricular block, left anterior hemiblock, and antero-septal pseudonecrosis. (B) Echocardiogram (on the left a sub-costal projection; on the right a short-axis projection obtained at the level of the papillary muscles): presence of a severe thickening of the walls of both ventricles in the absence of a dilation of the chambers, minimum pericardial effusion, 'granular sparkling' of the myocardium, increase in atrium-ventricular valve thickness and bi-atrial dilation. (C) Cardiac magnetic resonance (similar projections compared to echocardiography): presence of diffuse subendocardial accumulation of gadolinium (typical of an infiltrative process such as cardiac amyloidosis). (D) Scintigraphy with ^{99m}Tc-DPD: hyper-fixation of the tracer at myocardial level with extremely attenuated bone uptake (the accumulation is also visible at the level of the joints and carpal tunnel). (E) Histological analysis of endomyocardial biopsy respectfully (above) with haematoxylin-eosin (the amyloid deposits at extracellular level appear pale pink in comparison to the darker surrounding myocardial tissue) and (below) immuno-histochemical analysis positive for transthyretin (intense rust colour at interstitial level).

independent of the underlying aetiology; and in the more specific treatment for the different forms of amyloidosis, aimed at interrupting the formation of amyloid ('anti-amyloidogenic therapy').¹¹

Supportive therapy

It can be defined as 'symptomatic treatment' and is aimed at improving the patient's quality of life and includes strategies aimed at neurological manifestations (analgesic drugs for sensorimotor polyneuropathy, drugs to control the sphere of dis-autonomic problems and physiotherapy) but above all cardiac directed therapy.

Patients with AC tend to retain fluids and are very sensitive to sodium intake, whose daily use should be limited to 1.5-2 g by associating a regime of fluid restriction for a total of 1-1.5 L/day.

Diuretics are the mainstay of heart failure therapy in patients with AC. Loop diuretics (furosemide, torsemide, and bumetanide) are the most commonly used active ingredients, which can be associated with anti-aldosteron such as spironolactone or eplerenone.

The patient with AC needs frequent cardiology follow-up, at least every 6 months, to optimize the management of heart failure, with the ultimate aim of reducing hospitalization due to exacerbation of heart failure.

It is important to stress that patients with AC are more sensitive to the side effects of drugs normally used for the management of heart failure, which therefore must be administered with great care. In general, the use of digitalis/digoxin is highly discouraged: *in vitro*, a link between the drug and amyloid fibrils has been demonstrated which could alter the blood concentration of digitalis, with the risk of underestimating the dosage and causing toxicity. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers should be used with caution because of the hypotensive effect, and beta blockers, which can reduce both systolic and cardiac output (in the presence of a true restrictive pathophysiology, should be prescribed with caution or even avoided). Diastolic filling of the ventricle and stroke volume are fixed, so the increase in heart rate remains the only mechanism for increasing cardiac output. Verapamil and Diltiazem are also contraindicated, also due to the chronotropic insufficiency they can determine.

Midodrine (α -agonist) can be used in selected cases with severe symptomatic hypotension, particularly in patients with autonomic dysfunction or who use large amounts of diuretics (potentially hypotensive), even in the absence of evidence.¹⁵

In the presence of arrhythmias such as flutter and atrial fibrillation, setting up an oral anticoagulant therapy is recommended (there are no specific experiences with NAOs, but no reasons not to use them). However, it is good to consider that the severe atrial dysfunction associated with AC may predispose to increased thrombo-embolic risk even in the presence of sinus rhythm.¹⁶

Conduction delays that require pacemaker implantation are not uncommon, especially in patients with TTR amyloidosis. On the contrary, the role of the implantation of a defibrillator in primary prevention of sudden arrhythmic

death remains marginal (the almost exclusive cause of sudden death is electromechanical dissociation), just as the role of ventricular resynchronization and mechanical supports remains to be clarified.¹⁷

Anti-amyloidogenic therapy of ATTR

Although orthotopic liver transplantation (often 'domino') has represented the first effective strategy of 'surgical gene therapy' and continues to be an effective solution in patients with V30M mutation in the initial phase of the disease, in patients with cardiac involvement, deposition of intra-myocardial amyloid does not stop after liver transplantation probably due to a 'nest effect' exerted by the deposits themselves against the circulating TTR (produced by the new liver and therefore not mutated): in these cases, the possibility of a combined liver and heart transplant could be considered.¹¹ The isolated cardiac transplantation represents, exceptionally, a therapeutic option in the ATTRm with 'cardiogenic' mutations or in the ATTRwt. However, it can be considered in the rare cases with isolated cardiac involvement in patients below 65 years.

A very intense research is underway to develop drugs able to prevent the production of amyloid in both forms of TTR amyloidosis. The aetiological drug therapy aims to intervene at three levels (*Figure 2*):

- (1) Block of TTR synthesis, possible thanks to interference or blockage (silencing) of messenger RNA at the hepatic cellular level to prevent transcription of the protein itself.
- (2) Stabilization of the TTR tetramer, by the action of stabilizing molecules that carry out their function by occupying the binding sites for the thyroid hormones and preventing the fragmentation of the protein and the subsequent precipitation in fibrils.
- (3) Removal of amyloid deposits from tissues.

The field in which the research is at a more advanced stage is that concerning the stabilization of the circulating TTR with two principles already available on the market: Tafamidis¹⁸ (Vyndaqel) and Diflunisal.¹⁹ In particular, since 2011 Tafamidis is approved for the treatment of familial neuropathic forms due to a TTR mutation: its use appears to have slowed the progression of the neurodegenerative process and to have promoted the maintenance of autonomic functions, allowing patients to benefit for a longer period of good quality of life, due to the absence of major side effects related to therapy. The first phase 3 study dedicated to assessing the efficacy and safety of Tafamidis in patients with ATTR CM has recently been concluded and published.²⁰ The multicentre international randomized study enrolled 441 patients to receive Tafamidis (80 mg or 20 mg/day) or placebo for 30 months with a 2:1:2 ratio and considered the hierarchical combination of mortality for all causes and hospitalization due to cardiovascular causes as primary endpoint and as main secondary endpoints the distance to the 6-min walk test and the quality of life. Overall the Tafamidis reduced the primary endpoint in a statistically significant manner ($P=0.0006$); in particular,

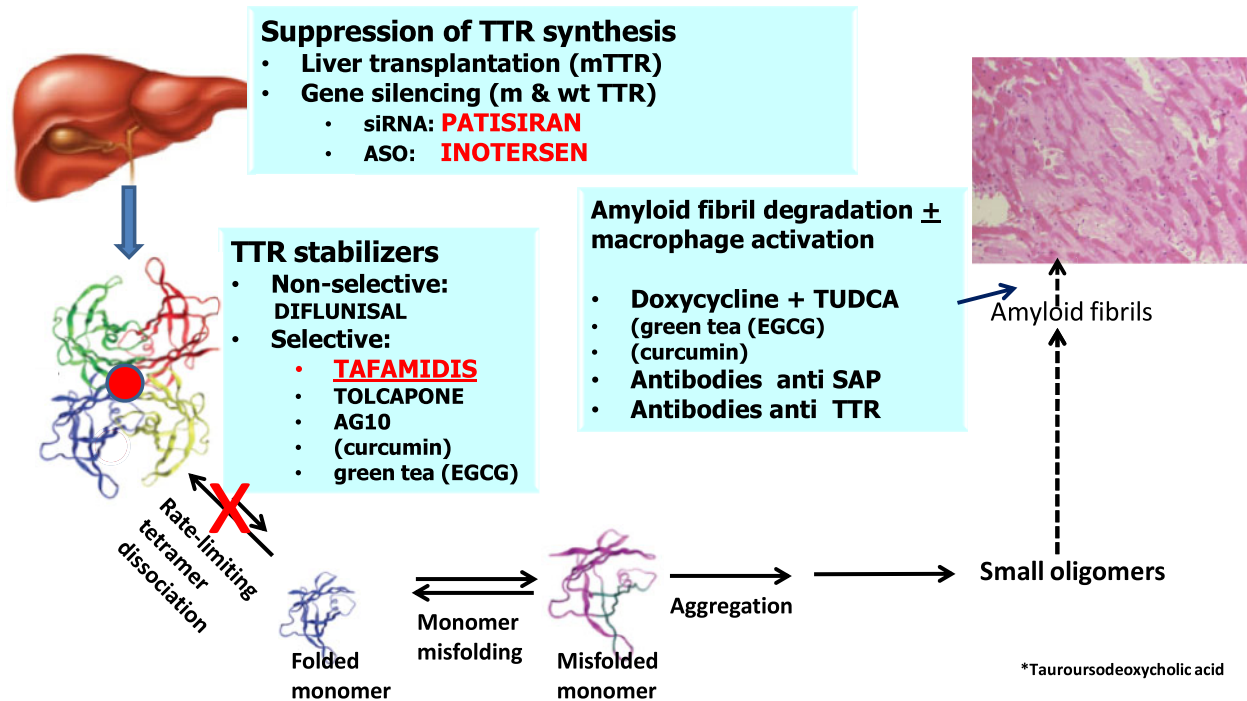


Figure 2 The different ‘disease modifying’ therapeutic strategies in ATTR are located in the respective therapeutic targets along the pathophysiological cascade leading to amyloidogenesis.

the relative mortality risk is reduced by 30% and that of hospitalization by 32%. The separation of the two actuarial curves (placebo and Tafamidis) occurs at about 18 months for survival and after about a year for hospitalizations. Furthermore, Tafamidis reduces the decline in both functional capacity to 6-min walk test and quality of life measured with the KCCQ-OS score. There are no treatment safety issues.

The analysis of the predefined subgroups shows a full agreement with the general results and no internal differences regarding the dose and aetiology (ATTRwt vs. ATTRm). As far as symptoms severity is concerned, patients with New York Heart Association III treated with Tafamidis showed a reduction in mortality but an increase in hospitalizations. In other words, the prolongation of survival induced by the drug (which obviously fails to affect the already advanced stage of the disease) exposes these patients to the risk of hospitalization for a longer period than placebo.

Both this observation and the late divergence of the curves underline the importance of starting the treatment as early as possible.

Conflict of interest: none declared.

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