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REVIEW

Oxidative damage in immunoglobulin light chain and transthyretin cardiac amyloidosis – a closer look

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Abstract

Heart failure is a progressive disease, representing a growing cause of morbidity, hospitalization, and mortality. An increasingly common type of heart failure with preserved ejection fraction (HFpEF) is an immunoglobulin light chain and transthyretin cardiac amyloidosis, in the pathophysiology of which oxidative damage appears to exert a strong impact. Reactive oxygen and nitrogen species have physiological signaling functions, but their overaccumulation, as in cardiac amyloidosis, leads to cardiomyocyte damage and apoptosis, and to cardiac hypertrophy and fibrosis. Moreover, such pathological processes worsen the redox damage with the perpetuation of an inflammatory state, in a vicious cycle. Here, the role of oxidative damage in the transthyretin and immunoglobulin light chain cardiac amyloidosis, the underlying pathogenic mechanisms, the therapeutic implications, and possible future strategies are reviewed.

Keywords: cardiac amyloidosis; cardiovascular; light chains; oxidative damage; transthyretin

Introduction

Among the wide variety of processes caused by or associated with oxidative damage, heart failure (HF) can be addressed with particular significance. Although reactive oxygen and nitrogen species have physiological signaling functions in the heart, a dysregulated presence of these molecules seems to promote a redox imbalance leading to cardiomyocyte damage (Sundaresan *et al.* 1995, Finkel 1998, Lambeth & Neis 2014). Indeed, cardiomyocyte stress disrupts intracellular redox homeostasis by increasing the reactive oxygen species (ROS)/reactive nitrogen species (RNS) steady-state concentration (Finkel 1998, Lambeth & Neis 2014). This leads to further reactive species production responsible for the damage of subcellular components. Mitochondrial dysfunction has often been found in HF, as it plays a key role in ROS and RNS production (Aimo *et al.* 2020).

HF is a complex, progressive disease characterized by the impairment of ventricular filling or systolic function, leading to symptoms such as dyspnea, fatigue, peripheral, or pulmonary edema (McDonagh *et al.* 2021). It represents a growing cause of morbidity and mortality and is altogether a common cause of hospitalization. The prognosis of patients with chronic HF is about 50% within 5 years of the initial diagnosis (McDonagh *et al.* 2021). The overall prevalence in the European adult population is estimated to be 2%, growing exponentially with age.

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Within the group of diseases leading to HF, particularly HF with preserved ejection fraction (HFpEF), cardiac amyloidosis (CA) is highlighted by the likely important role that oxidative stress has in its pathogenesis and disease progression. Its main feature is the accumulation of amyloid fibrils in the myocardium, most frequently formed from misfolded immunoglobulin light chains or transthyretin, both altering the cardiomyocyte redox balance.

This review aims to elucidate the actual impact of oxidative stress in the pathogenesis of light chain immunoglobulin and transthyretin cardiac amyloidosis, AL and ATTR respectively, with at the end a glance at the therapeutic implications.

Heart failure

HF is classified into three phenotypes based on the left ventricular systolic function measured with the left ventricular ejection fraction (LVEF):

(1) HF with reduced LVEF (HFrEF) is defined as an LVEF ≤ 40%.
(2) HF with mildly reduced LVEF (HFmrEF) is defined by an LVEF between 41% and 49%.
(3) HF with preserved LVEF (HFpEF) includes patients with an LVEF ≥ 50% and symptoms and signs of HF, with evidence of structural and/or functional cardiac abnormalities and/or raised natriuretic peptides (NPs).

HFrEF has long been considered the most frequent form, with its pathology is well-known and often characterized by an inflammatory response and oxidative damage resulting from primary cardiac insult, peripheral tissue hypoperfusion, or pressure and volume overload (McDonagh *et al.* 2021).

Interestingly, HFpEF can arise from a combination of risk factors and comorbidities, like aging, female gender, obesity, systemic arterial hypertension, atrial fibrillation, diabetes mellitus, chronic kidney disease, anemia, iron overload, obstructive sleep apnea syndrome , and chronic obstructive pulmonary disease (Pieske *et al.* 2019, McDonagh *et al.* 2021). Notably, the large majority of these comorbidities are associated with oxidative stress and inflammation, two conditions steadily associated with HFpEF as well. Furthermore, HFpEF can originate from infiltrative cardiomyopathies, such as amyloidosis, sarcoidosis, hemochromatosis, Fabry's disease, and glycogen storage disorders (McDonagh *et al.* 2021).

Light chain and transthyretin cardiac amyloidosis

In the context of HFpEF, CA seems to be a disease characterized by a dysregulated inflammatory response and ROS imbalance.

Amyloidosis is a group of multi-organ disorders caused by the extracellular deposition of proteolysis-resistant misfolded and insoluble proteins. Different precursor proteins can turn into amyloid fibrils, altering tissue structures and leading to various clinical phenotypes related to the specific protein infiltration (Lachmann & Hawkins 2006).

Light chain amyloidosis (AL) and transthyretin amyloidosis (ATTR) are the two forms of AL with greater cardiac involvement, which are often the main cause of morbidity and mortality, regardless of the underlying cause of amyloid accumulation (Martinez-Naharro *et al.* 2018). In Table 1, we summarize their main features. Although ATTR amyloidosis was considered a rare disease in the past, advanced imaging techniques and non-invasive diagnostic methods are leading to increasingly frequent diagnoss of this entity.

The prevalence of CA was studied in asymptomatic and symptomatic subjects and in autopsy series (Aimo *et al.* 2022). Approximately 0.2–0.5% of patients undergoing bone scintigraphy for non-cardiac reasons were suspected of ATTR-CA (Longhi *et al.* 2015, Mohamed-Salem *et al.* 2018, Bianco *et al.* 2021, Cuscaden *et al.* 2021). Endomyocardial biopsies performed in patients with HFpEF of unclear etiology showed an overall CA prevalence of 14% (Hahn *et al.* 2020). Autopsies performed in unselected elderly individuals >85 years of age demonstrated the presence of amyloid in approximately 25% of hearts, despite the absence of established cardiomyopathy. ATTR-CA was the most frequently diagnosed form (Mohammed *et al.* 2014, Aimo *et al.* 2022, Paraskevaidis *et al.* 2023). The prevalence of CA increases with age, while female sex seems to be a protective factor (Aimo *et al.* 2022).

AL amyloidosis is caused by a plasma-cell dyscrasia with misfolded immunoglobulin light chains deposited in multiple organs, including the heart in half of the affected patients. Extracardiac tissue infiltration leads to proteinuria and nephrotic syndrome, peripheral neuropathy or autonomic dysfunction, gastrointestinal motility disorders, hepatomegaly, periorbital purpura, and macroglossia.

ATTR amyloidosis is caused by the accumulation of misfolded transthyretin, a protein produced mainly by the liver that participates in transporting thyroid hormone and retinol. No relationship between thyroid disorders and amyloidosis was found (Zanotti & Berni 2004, Gertz *et al.* 2015*a*). The structure of transthyretin is due to DNA mutations in the case of the hereditary ATTR (hATTR), while in the wild-type ATTR (wtATTR), the ATTR tetramer misfolding is favored by the normal aging process (Plante-Bordeneuve 2003, Hellman *et al.* 2008).

Non-hereditary wild-type TTR amyloidosis is often a senile disease with a strong male predominance (Connors *et al.* 2016) and a relatively late diagnosis because of its gradual progression. The heart is the most affected

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Table 1 Main features of the light chain immunoglobulin and transthyretin amyloidosis.

	AL amyloidosis	TTR amyloidosis	
Subtypes	Light chain amyloidosis (AL)	Hereditary TTR amyloidosis (hATTR)	Wild-type TTR amyloidosis (wtATT)
Precursor	Immunoglobulin FLC	TTR protein	TTR protein
Pathogenesis	Infiltration of a small plasma cell clone into bone marrows	Destabilization and misfolding of native TTR into fibrils of amyloid aggregates	
	→ Production of amyloidogenic monoclonal immunoglobulin free light chains	→ Point mutations enhancing the instability of TTR structure	→ Ageing-associated failure of repair and proteostatic mechanism
	Nonhereditary	Autosomal dominant	Non-hereditary
	Chromosomal abnormality t(11;14) translocation	150 variants (Val50Met)	
Age	50–90 years	30–50 years	>70 years
Sex	Male = female	More severe in males	Male >> female
Clinical manifestations	Multiorgan disease	Mutation-related phenotype	Mainly asymptomatic Often undiagnosed
Cardiac involvement	Direct proteotoxicity of FLC	Tissue structure alteration	
	Greater myocardial amyloid fibril burden	Wall thickening in both ventricles	
	Worse systolic dysfunction	Hypertrophic cardiomyopathy	
		Early stage → Diastolic dysfunction	
		Late stage → Systolic dysfunction	
		Supraventricular arrhythmia	
		Atrial fibrillation	
		Atrial flutter	
		Conduction disorder	
		Atrioventricular conduction delay	
Typical extracardiac involvement	Peripheral nerves	Peripheral neuropathy, autonomic dysfunctions	Musculoskeletal disorders
	Kidney	Nephropathy	Bilateral carpal tunnel syndrome
	Liver		Lumbar spinal stenosis
	Gastrointestinal tract		Biceps tendon rupture
	Skin and soft tissue		

FLC, free light chains; TTR, tranthyretin.

organ, while systemic involvement is rare except for some musculoskeletal disorders such as bilateral carpal tunnel, lumbar spinal stenosis, and biceps tendon rupture (Nakagawa *et al.* 2016).

Hereditary TTR amyloidosis (hTTR) is caused by mutations in the TTR gene, often following an autosomal dominant pattern of inheritance. More than 100 missense mutations can predispose greater instability of transthyretin and its misfolding, leading to an earlier onset of the disease (Gopal *et al.* 2019). Specific mutations, together with epigenetic factors, determine the different onset timing, geographical distribution, prognosis, and the predominant cardiac rather than neurological phenotype, with peripheral neuropathy and autonomic dysfunctions being the most common clinical manifestations besides cardiac involvement (Gertz *et al.* 2015*a*). Specific hATTR genotypes, such as Val122Ile, are associated with a poorer prognosis (Singh *et al.* 2017).

Oxidative stress and inflammation in the myocardium: a vicious cycle that could lead to HF

Under physiological conditions, a significant bulk of reactive chemical species is generated within eukaryotic cells, including leukocytes and endothelial cells, as key intermediates in a variety of biochemical (e.g. mitochondrial respiration pathways) and biological (e.g. phagocytosis) reactions of importance. The principal reactive oxygen species include superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), and nitric oxide (NO). The generation of ROS and RNS results from oxidation–reduction reactions driven by transitional metal catalytic interactions or by specific enzymes such as NADPH oxidases (NOXs), dual oxidase (DUOX), xanthine oxidase, and nitric oxide synthase (Fig. 1) (Halliwell & Gutteridge 2015).

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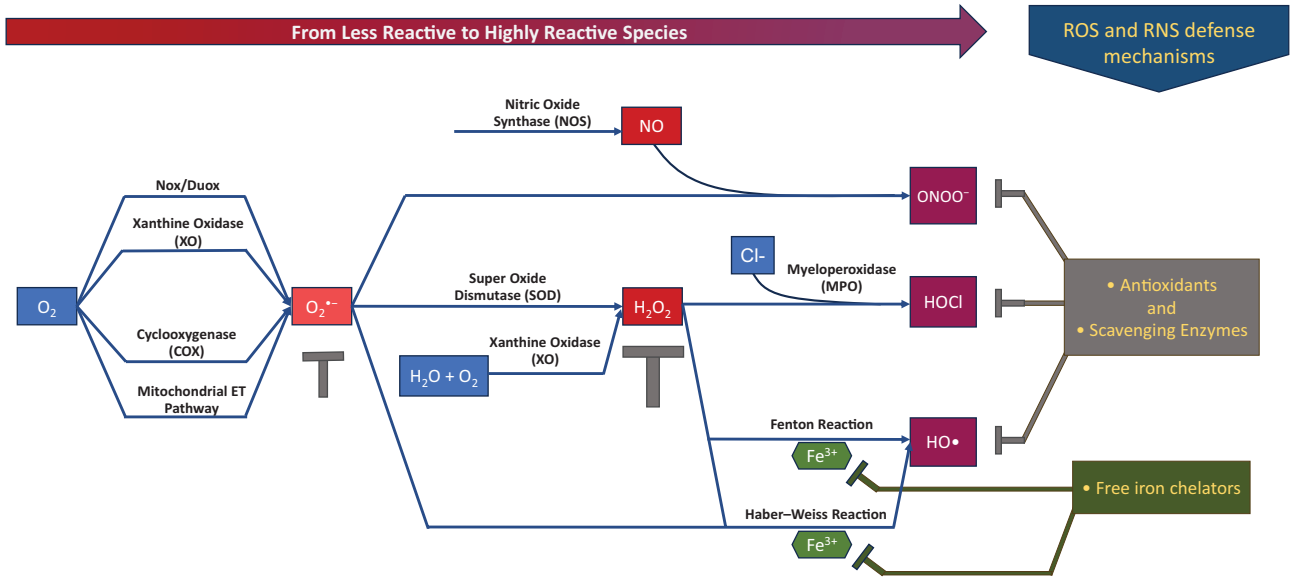

Figure 1

Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The color spectrum of the arrow bar above the scheme varies with the reactivity of these species. The main classes of defense mechanisms against ROS and RNS are depicted briefly. ET, electron transport; $O_2^{\bullet-}$, superoxide ion; H_2O_2, hydrogen peroxide; NO, nitric oxide; Cl^-, chloride ion; $ONOO^-$, peroxynitrite; HOCl, hypochlorous acid; $OH^\cdot$, hydroxyl radical; Fe^{3+}, ferric ion.

The critical point is maintaining an optimal, namely, physiological, quantity of ROS/RNS within cells and tissues, through a delicate and dynamic balance between their production and degradation rates. Light chain immunoglobulin has indeed developed a pleiotropic and ubiquitous defense system in eukaryotes to preserve intracellular homeostasis by avoiding an excessive production and/or a diminished removal of ROS/RNS, namely, a condition termed oxidative stress (Fig. 2) (Halliwell & Gutteridge 2015, Losada-Barreiro *et al.* 2022).

An oxidative stress condition can deregulate the inflammatory response and stimulate apoptosis through both extrinsic and intrinsic pathways, as well as induce fibrosis in many tissues and organs including the myocardium (Park *et al.* 2001). Fibrosis can occur through the action of growth factors, such as transforming growth factor-β (TGF-β) and connective tissue growth factor, and through the influence that the renin–angiotensin–aldosterone system has on fibroblast differentiation (Fujii *et al.* 2005, Liu & Desai 2015). A sustained increase of ROS levels, as a consequence of chronic inflammation, could heavily affect heart physiology in different ways. Oxidative stress could interfere with intracellular calcium homeostasis mainly through the ryanodine receptor 2 (RyR2) and ROS-activated calmodulin-dependent protein kinase II (CaMKII) (Zima & Blatter 2006, Johnston *et al.* 2015). In addition, ROS disproportion leads to ion imbalance, promoting arrhythmias (Jeong *et al.* 2012). Overexpression of NOX, an important enzyme in physiological vasculogenesis, seems to play a major role in the cardiac hypertrophy mechanism (Kuroda *et al.* 2010, Zhang *et al.* 2010).

Moreover, HF features such as neurohormonal activation, pressure–volume overload, and altered cardiomyocyte metabolism play a fundamental role in the perpetuating an inflammatory state, leading to a vicious cycle that is difficult to stop. This is revealed by the high levels of inflammatory mediators found in patients suffering from HF, such as tumor necrosis factor-α (TNF-α) (Higuchi *et al.* 2002) and interleukin 6 (IL-6) (Ridker & Rane 2021, Alogna *et al.* 2023). Moreover, comorbidities such as diabetes mellitus, overweight, and obstructive sleep apnea promote systemic inflammation, exacerbating the myocardial ROS imbalance (Kalogeropoulos *et al.* 2010, Paulus and Tschöpe 2013, Franssen *et al.* 2016).

ROS binding to NO decreases its circulating levels, leading to lower guanylate cyclase activation with consequent lower cyclic guanosine monophosphate (cGMP) production and cGMP-protein kinase G (PKG) downregulation. cGMP-PKG regulates cardiomyocyte contractility through cellular calcium homeostasis, suppresses hypertrophic changes, and favors ventricular diastolic relaxation by phosphorylation of troponin I and titin. Notably, the pattern of lower levels of NO and myocardial PKG hypoactivity is more frequent in HFpEF than in HFrEF (Francis *et al.* 2010, Mongirdienė *et al.* 2022). Besides involving NO, ROS also directly activates the TGF-β/Smad3 pathway, promoting myocardial fibrosis (Michels da Silva *et al.* 2019).

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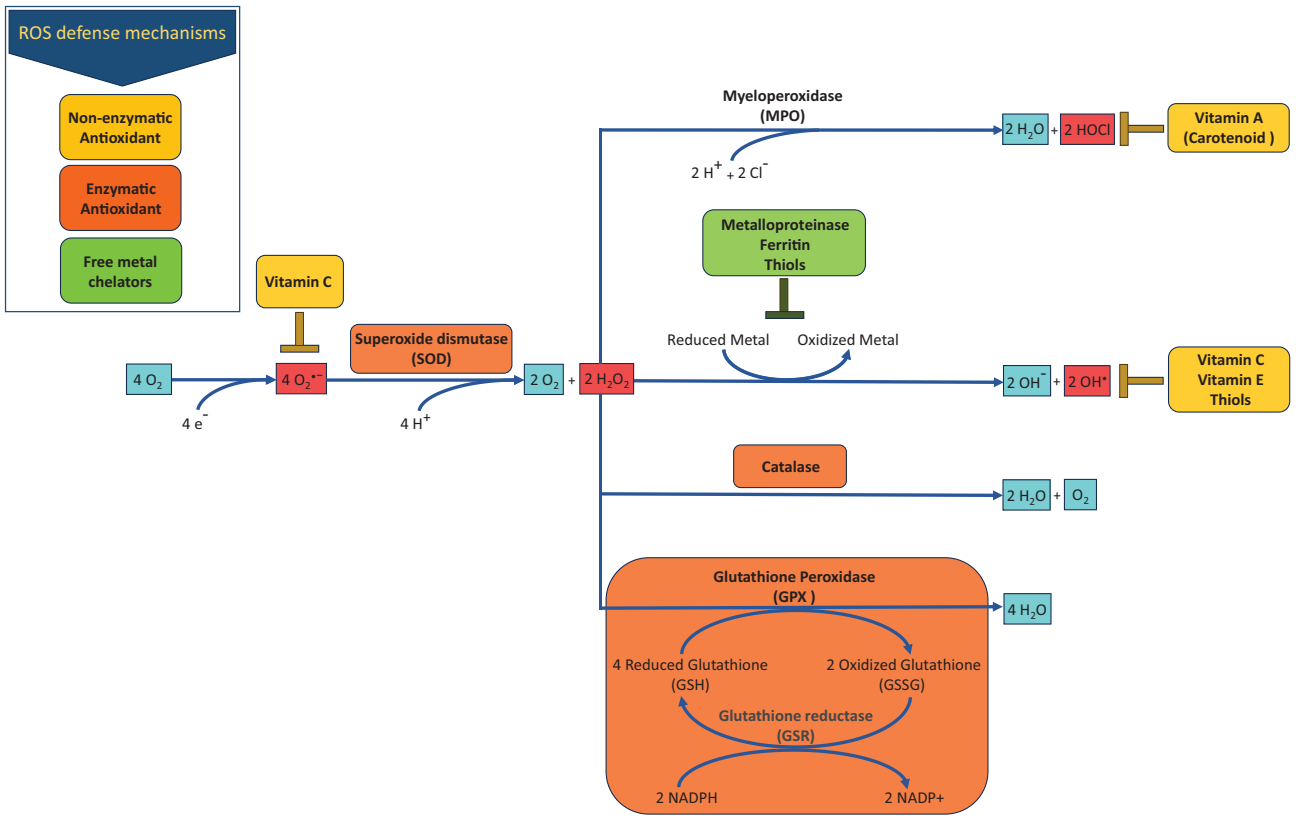

Figure 2

The molecular defensive system against ROS, in detail. $O_2^{\bullet -}$, superoxide ion; H_2O_2, hydrogen peroxide; NO, nitric oxide; $ONOO^-$, peroxynitrite; HOCl, hypochlorous acid.

Oxidative stress and damage at the cardiac level in AL and ATTR amyloidosis

Amyloid fibril infiltrations in the heart lead to disruption of tissue structure, biventricular wall thickening, and stiffness with consequent remodeling, elevated diastolic filling pressures with occurrence of HFpEF, atrial dilation, and supraventricular arrhythmias arising from either atrial fibrillation or atrial flutter (Merlini & Bellotti 2003). Microvascular amyloid infiltration was shown to reduce myocardial perfusion. The conduction system is also involved, with atrioventricular conduction delays.

The simple mechanical displacement of normal parenchymal tissue cannot sufficiently explain the cardiac dysfunction observed in both AL and ATTR amyloidosis. Misfolded light chain immunoglobulin, altered/misfolded transthyretin, and amyloid fibrils are believed to play the primary and crucial role in tissue toxicity (Quarta *et al.* 2014).

Both immunoglobulin light chain and transthyretin-related amyloidosis are steadily associated with oxidative stress, and such an unbalanced redox status appears heavily involved in the pathogenesis of these disease processes (Gertz *et al.* 2015*b*). In fact, several experimental data strongly support a significant role of these two types of misfolded proteins in inducing an excessive accumulation of ROS in cardiomyocytes (Sharma *et al.* 2019).

With regard to the pro-oxidant property of abnormal deposition of misfolded immunoglobulin light chain in the myocardium, a marked increase of ROS, as detected by using the 2',7'-dichlorodihydrofluorescein-diaceta te reagent (DCFDA), and a net overexpression of the heme oxygenase 1 enzyme (HO-1), a reliable marker of adaptation to cell oxidative challenge, was observed in rat ventricular cardiomyocytes incubated for 24 h in the presence of light chain immunoglobulin isolated from patients with cardiac light chain immunoglobulin amyloidosis, while an identical cell treatment with light chain immunoglobulin derived from non-amyloid myeloma patients did not lead to a rise of the two oxidative stress markers (Brenner *et al.* 2004). The unbalanced redox status toward oxidation was accompanied by cardiomyocyte damage, dysfunction, and apoptotic death, changes that were prevented by suitable cell preincubation with the antioxidant superoxide dismutase/catalase mimetic manganese(

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III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP) (Brenner *et al.* 2004).

A source of intracellular overproduction of ROS in AL amyloidosis appears to be the membrane NADPH oxidase enzyme (NOX), the isoforms of which NOX2 and NOX4 are mainly represented in cardiomyocytes. In fact, the NOX-triggered cell signaling driven by p38 MAPK pathway (Santillo *et al.* 2015) was demonstrated to be upregulated in the cardiomyocytes challenged with light chain immunoglobulin derived from patients with cardiac AL (Shi *et al.* 2010). In parallel, the changes observed in the so-treated cardiomyocytes were significantly quenched by pretreatment with the antioxidant MnTMPyP or the selective p38 inhibitor SB203580, thus indicating that oxidative stress may act as a primary cause of cardiac damage and malfunction (Shi *et al.* 2010).

Of note, p38 MAPK simultaneously plays a role in the transcription of brain natriuretic peptide (BNP). This confirms the association of cardiotoxic light chain action and MAPK signaling activation with elevated BNP levels (Shi *et al.* 2010). The level of abnormally circulating light chains might be useful in formulating the clinical prognosis in patients with AL, as it also corresponds with cardiac biomarker elevations (Kumar *et al.* 2012). Moreover, specific chemotherapy aimed at reducing the underlying clone, thus the quantity of circulating amyloidogenic free light chains (FLC), was also seen to reduce BNP levels, regardless of the amount of amyloid deposition in the myocardium (Wechalekar *et al.* 2022*a*).

A second source of ROS that is most likely upregulated in AL is the cardiomyocyte mitochondria. In the *Caenorhabditis elegans* treated with light chain immunoglobulins from patients with CA, the impairment of the contraction–relaxation rhythm of the pharyngeal muscle appeared to be dependent on a marked mitochondrial oxidant burst as detected by MitoSOX Red staining, since not inhibited by pretreatment with antioxidants like N-acetyl cysteine, ascorbic acid, and epigallocatechin gallate (EGCG). The mitochondrial burst was not observed when the *C. elegans* pharyngeal pumping was treated with light chain immunoglobulins from nonamyloidogenic multiple myeloma patients (Diomede *et al.* 2014). The same group then demonstrated that the mitochondrial ROS burden observed in *C. elegans* was dependent on the presence of metal ions, particularly copper (Diomede *et al.* 2017).

As regards transthyretin-related amyloidosis, the tetrameric protein TTR accumulates in the extracellular spaces of various organs including the heart, then dissociates into monomers that misfold and aggregate to generate oligomers and amyloid fibrils (Wu & Chen 2024). Both entities are provided with remarkable cytotoxicity because of their pro-apoptotic properties and overproduction of ROS and RNS (Sharma *et al.* 2019). Similar to the pro-oxidant mechanisms involved in the cardiotoxic effects of misfolded light chain immunoglobulin, in the case of misfolded/aggregated TTR, the two main sources of ROS accumulation in cardiomyocytes were identified in upregulated NOX and altered mitochondrial bioenergetics. In the murine cardiac muscle cell line HL-1 overloaded with TTR aggregates, the binding of the aggregates to the cardiomyocyte surface and the subsequent internalization of oligomers were demonstrated. Consequently, an overproduction of ROS occurred, detected by the DCFDA fluorescent probe method, due to a marked derangement of mitochondrial membrane potential (Sartiani *et al.* 2016). Such oxidative stress state condition was accompanied by a net change in intracellular calcium homeostasis with ensuing cardiac muscle cell dysfunction (Sartiani *et al.* 2016).

The involvement of NADPH enzyme, as another main source of ROS, was demonstrated in human neutrophils showing an oxidative burst following the treatment with amyloid fibrils derived from aggregated TTR. The cell preincubation with the NADPH selective inhibitor diphenyleneiodonium prevented the induction of ROS production, as detected by using the probe dihydrorhodamine 123 (Azevedo *et al.* 2012) (Azevedo *et al.* 2012).

The enhanced formation of ROS by aggregated TTR was shown to be paralleled by an overproduction of RNS, as measured in terms of nitrite and nitrate levels, in two human cell lines, namely, the epidermoid A431 and the Schwannoma sNF943 cell lines (Fong & Vieira 2013). The same authors later reported an increased concentration of hydrogen peroxide paralleled by a partial inactivation of catalase and a diminished level of reduced glutathione (GSH) in both epidermoid and Schwannoma cell lines when treated with aggregated TTR (Fong *et al.* 2017).

RNS, mainly generated by the endothelial nitric oxide synthase, might contribute to the oxidative damage observed in CA, as some amyloidogenic variants were proven to become pathogenic after undergoing S-nitrosylation. The nitrosylation of the TTR oligomers seems to enhance the ability to form amyloid fibrils with consequent increased oxidative damage, leading to a vicious cycle (Saito *et al.* 2005).

In addition to the 'consumption' of antioxidant defense elements like catalase and reduced glutathione as a consequence of the sustained oxidative stress condition determined by deposition in the heart, as in other organs, of aggregated TTR, the misfolded protein was shown to otherwise affect the antioxidant system. The TTR tetrameric protein, which usually binds two molecules of thyroxine and two retinol–retinol-binding protein complexes, in condition of diminished availability of retinol (vitamin A), like sustained oxidative stress, exhibits its cryptic protease function and cleaves Apolipoprotein A1, thereby hampering the antioxidant activity of high-density lipoproteins (Liz *et al.* 2004). Further, GSH could bind to thiol residues of TTR, that is this protein becomes glutathionylated, thereby sequestering some of the antioxidant tripeptide (Sharma *et al.* 2019). Figure 3 shows the main sources of ROS and RNS, as well as relevant causes of antioxidant depletion in AL and ATTR amyloidosis.

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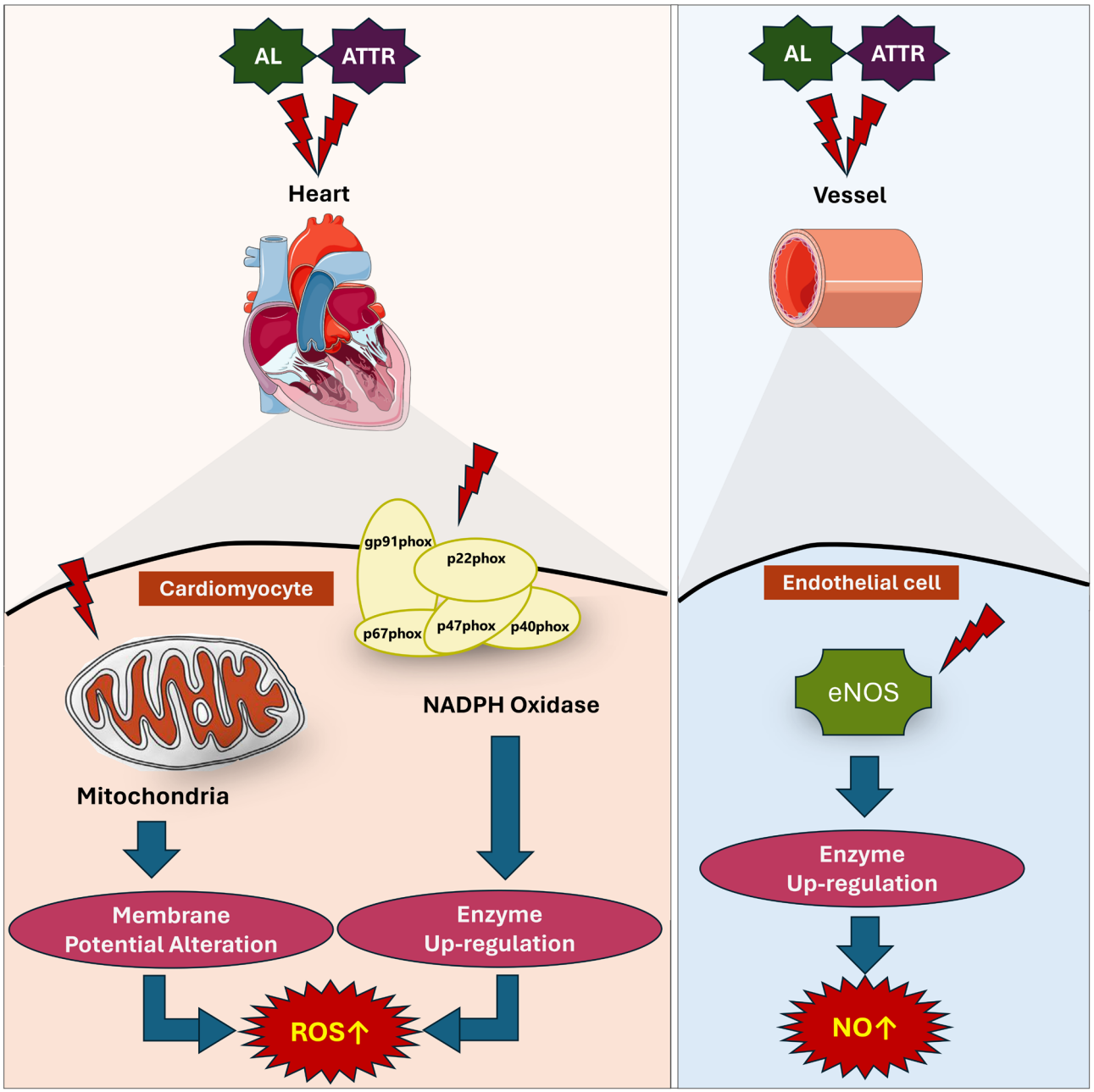

Figure 3

Main cellular and molecular sources of ROS and RNS in AL and ATTR amyloidosis. gp91phox/p22phox/p40phox/p47phox/p67phox, subunits of NADPH oxidase (NOX); eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; NO, nitric oxide.

In the case of ATTR amyloidosis, it is noticeable that tissue dysfunction occurs even before TTR fibril deposition in tissue. This indicates a non-intermediary toxic action of the prefibrillar protein. In laboratory trials, small TTR intermediates (monomers and oligomers smaller than 100 kDa) cause cytotoxicity through interactions with membrane proteins and cholesterol. The consequential activation of apoptotic mechanisms through cleavage of caspase 3/7 and the rise of ROS by means of superoxide formation can justify, at least partially, cardiac dysfunction (Reixach *et al.* 2004, Manral & Reixach 2015).

Moreover, in individuals with ATTR amyloidosis, comorbidities such as diabetes, hypertension, chronic kidney disease, and atrial fibrillation contribute independently to impaired ventricular relaxation and promote a pro-oxidative state, accelerating amyloid deposition. This could be an explanation of the

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heterogeneous clinical manifestations and different progression timing in mutant TTR carriers.

In Fig. 4, the possible consequences of the oxidative damage occurring in HFpEF are depicted.

AL and ATTR amyloidosis: from pathophysiology to therapeutic targets

At present, treatment of CA mainly consists of therapies based on general guidelines for the management of HF and arrhythmias.

Treatment of the hematological disease underlying AL

Specific therapy for AL amyloidosis aims at suppressing FLCs by treating the underlying hematological disease, therefore reducing the number of light chain-producing clones.

Low-risk patients are eligible for high-dose melphalan administrations followed by autologous stem cell transplantation (ASCT) (Dispenzieri *et al.* 2004, Sher *et al.* 2016). Hematological response after treatment was shown to reduce organ damage and improve prognosis in 65–80% of patients.

Patients with organ damage (i.e. NYHA (New York Heart Association) class III–IV, increased troponin T, arterial

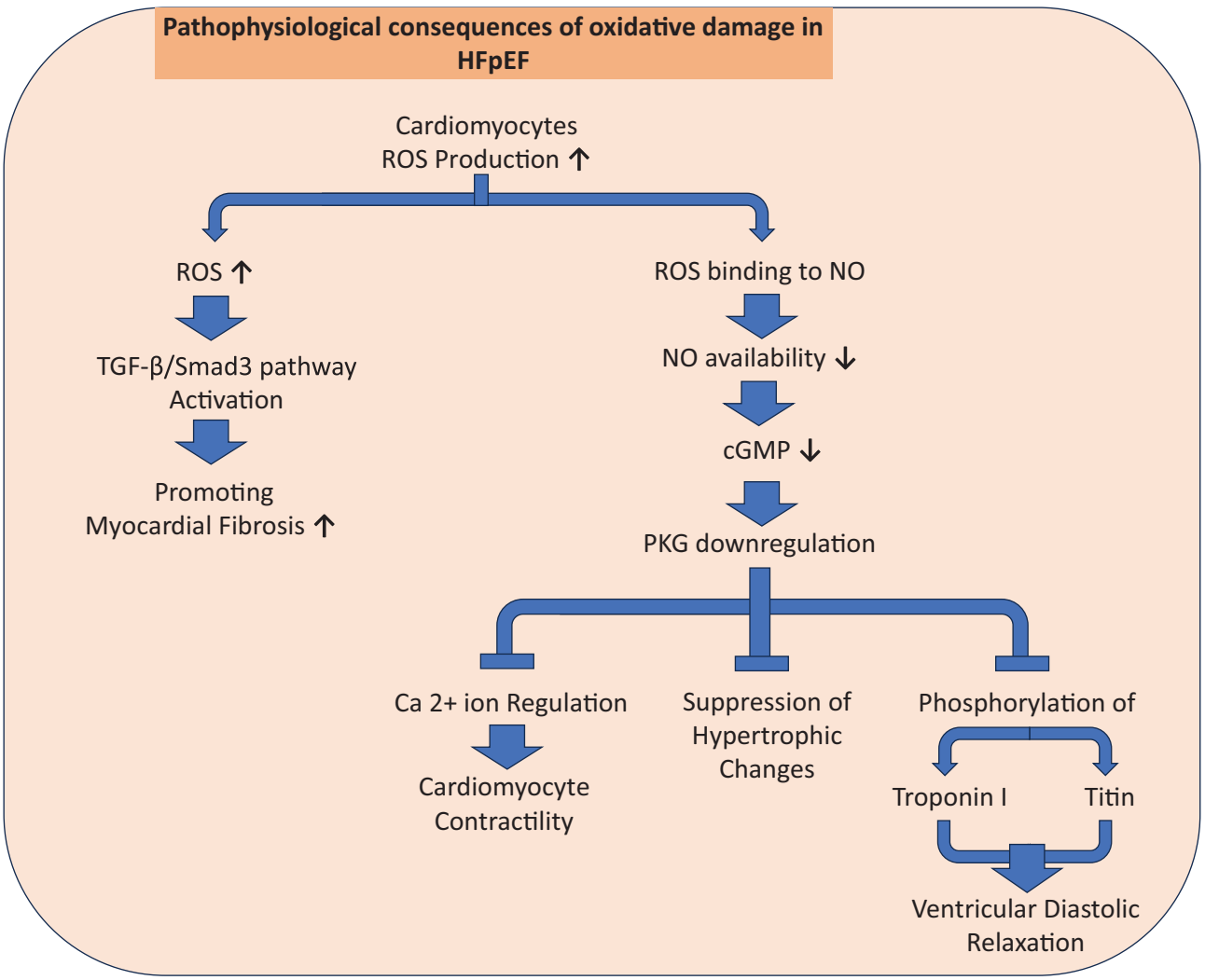

Figure 4

Pathophysiological consequences of oxidative damage in heart failure with preserved ejection fraction. HFpEF, heart failure with preserved ejection fraction; ROS, reactive oxygen species; NO, nitric oxide; TGF-β, transforming growth factor β; Smad3, small mother against decapentaplegic 3; cGMP, cyclic guanosine monophosphate; PKG, cGMP-dependent protein kinase.

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hypotension, renal failure) are not eligible for ASCT. In this setting, treatment regimens are BMDex (melphalan + dexamethasone + bortezomib) (Kastritis *et al.* 2020), CyBorD (cyclophosphamide + bortezomib + dexamethasone). In this setting, Dara-CyBorD (CyBorD + daratumumab, a human CD38-targeting antibody) recently proved to be effective (Kastritis *et al.* 2021).

Regarding ATTR amyloidosis, orthotopic liver transplantation had been the only treatment option for decades. Although it was proven successful in patients with familial amyloid polyneuropathy (FAP), it was not effective in reducing the organ damage caused by the amyloid fibrils already deposited in tissues, nor the misfolding of circulating proteins caused by them. Therefore, several disease-modifying treatments were developed for ATTR CA, following three main mechanisms, as illustrated in Fig. 5:

- The stabilization of TTR tetramers, in order to reduce the formation of amyloid fibrils (tafamidis, diflunisal, acoramidis).
- The reduction of amyloid production through silencing the transthyretin gene (patisiran, vutrisiran, inotersen, eplotersen).
- The reabsorption and degradation of already deposited fibrils in tissues by using monoclonal antibodies.

Tafamidis

Tafamidis is a 5-benzoxazole derivative that binds to transthyretin with high affinity and selectivity, hindering tetramer dissociation and consequent amyloidogenesis. It was proven to reduce all-cause mortality, the number of HF admissions, and the hospitalization days and improve the functional class in both cardiac and non-cardiac hereditary and wtTTR-CA with positive biopsy, mainly in NYHA class I and II groups at baseline (Maurer *et al.* 2018, McDonagh *et al.* 2021). Its greater limit is the cost-effectiveness.

Acoramidis

With the same premises, a safe and effective alternative is represented by the recently introduced Acoramidis (AG10), a strong and selective tetramer stabilizer of both mutated and wild-type TTR. In the latest ATTRibute-CM trial, held on ATTR-CA patients with NYHA I–III HF, it was shown to improve cardiovascular-related hospitalizations, NT-proBNP levels, and quality of life, thus representing a safe and effective alternative (Gillmore *et al.* 2024).

Diflusinal

Diflunisal also stabilizes the transthyretin tetramer, preventing its misfolding. It demonstrated slowing the progression of polyneuropathy in hATTR patients and improving laboratory and echocardiographic parameters in cardiac hATTR amyloidosis. However, being a nonsteroidal anti-inflammatory drug, it carries gastrointestinal side effects and nephrotoxic properties, limiting its chronic administration in the elderly population (Berk *et al.* 2013).

Patisiran

Patisiran is a siRNA molecule, administered intravenously, that specifically inhibits hepatic synthesis of transthyretin, lowering its circulating levels. It was approved for the treatment of patients with FAP regardless of the presence of cardiac involvement, showing halting or reversion of disease progression and an improvement of neuropathy in patients with hereditary transthyretin amyloidosis, together with improvements in quality of life, walking, nutritional status, and activities of daily living (Adams *et al.* 2018).

Vutrisiran

A second siRNA, vutrisiran, was recently approved, showing similar efficacy to patisiran, good tolerability, and better ease of use, as it can be administered subcutaneously (Adams *et al.*, 2023).

Inotersen

Inotersen is an antisense oligonucleotide that cleaves the mRNA of the TTR gene, leading to its degradation. It is approved to slow the progression of neurological amyloidosis. Its side effects are thrombocytopenia and glomerulonephritis (Dasgupta *et al.* 2020).

NTLA-2001

CRISPR–Cas9-based *in vivo* gene editing is a promising treatment option for amyloidosis. Preliminary findings demonstrated that the administration of NTLA-2001 to patients with hATTR amyloidosis with polyneuropathy was associated with sustained reductions in the serum TTR protein concentration (Gillmore *et al.* 2021).

Organ transplantation

Liver and cardiac transplantation can be considered in the end-stage phase of familial aTTR cardiac amyloidosis.

Tools for degradation of amyloid fibrils

Tauroursodeoxycholic acid (TUDCA) is a biliary acid that reduces non-fibrillar TTR aggregates but needs further research (Cardoso & Saraiva 2006, Cardoso *et al.* 2010, Teixeira & Saraiva 2015). Anti-TTR monoclonal antibodies and anti-serum amyloid P component (SAP) antibodies

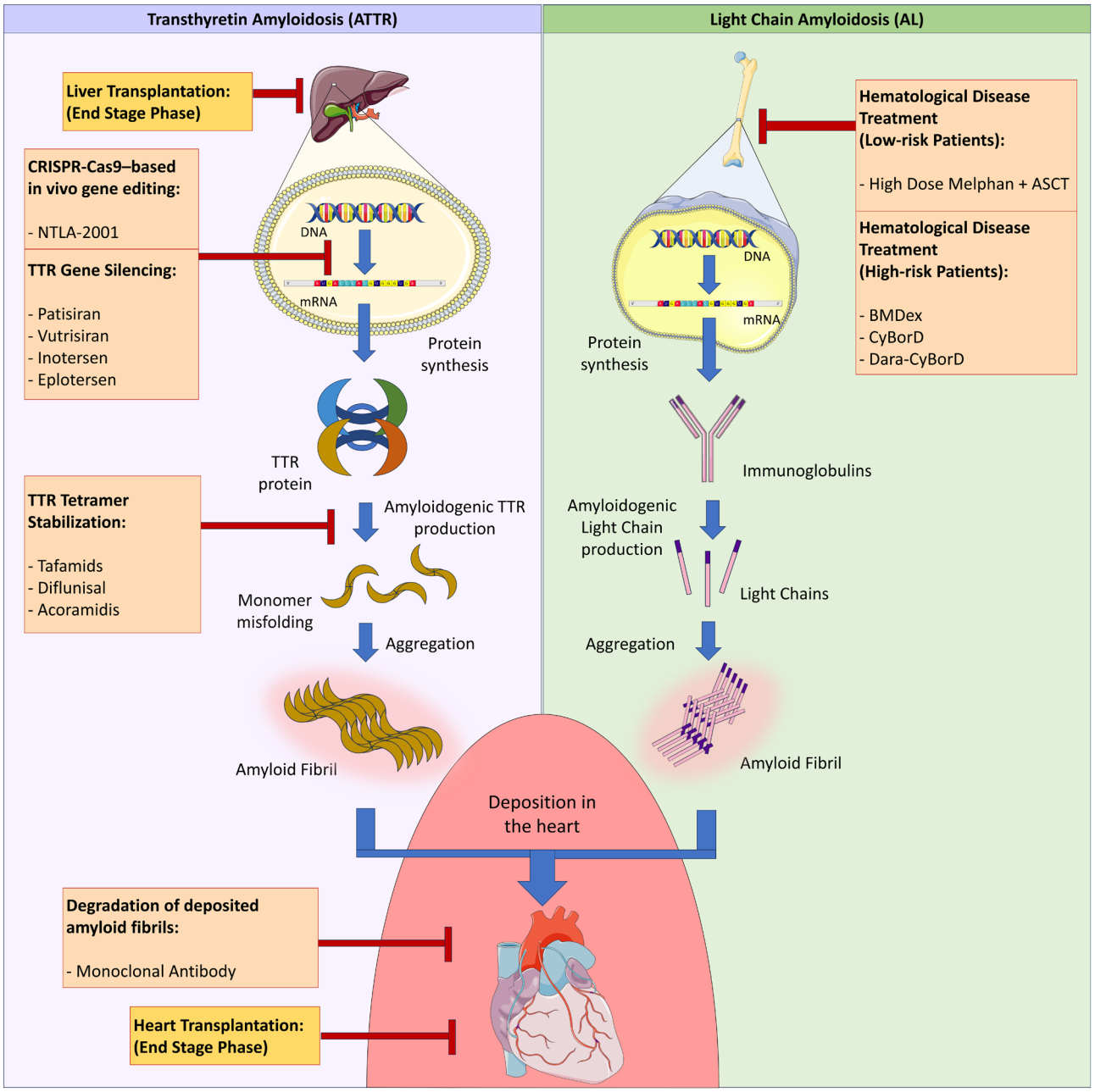

Figure 5

Therapeutic strategies in amyloidosis. CRISPR, clustered regularly interspaced palindromic repeats; Cas9, CRISPR-associated protein 9; NTLA-2001, an *in vivo* gene-editing therapeutic agent; TTR, transthyretin; ASCT, autologous stem cell transplant; BMDex, melphalan + dexamethasone + bortezomib; CyBorD, cyclophosphamide + bortezomib + dexamethasone; Dara-CyBorD, cyclophosphamide + bortezomib + dexamethasone + daracimumab; TTR, transthyretin.

(e.g. dezamizumab) have been studied with the aim of promoting the phagocytosis of TTR amyloid aggregates, but they have not yielded successful results so far (Phay *et al.* 2014, Higaki *et al.* 2016, Hosoi *et al.* 2016, Galant *et al.* 2016, Richards *et al.* 2022, Wechalekar *et al.* 2022*b*).

In Fig. 5, the present therapeutic strategies in amyloidosis are depicted.

ROS quenching and anti-inflammatory approaches

Flavonoids

Among the inhibitors of amyloid substance formation is the family of flavonoids, a subgroup of polyphenols,

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pleiotropic compounds also provided with strong antioxidant properties. Their aromatic rings enable the prevention of amyloid fibrils aggregation by creating non-covalent bonds to the core structure of the amyloid proteins (Miyata *et al.* 2010). Generally, flavonoids have been found to stabilize the TTR tetramers; however, their effects vary among the family. The flavonoid EGCG is a natural compound found in green tea. It showed significant efficiency in the inhibition of TTR amyloid aggregation by binding the tetrameric non-misfolded transthyretin and stabilizing its structure while preserving its physiological function, including the T4 transportation. In addition, a potential role in the disaggregation of amyloid deposits and disruption of preformed fibrils was proposed, leading to a conversion into unstructured off-pathway oligomers. The large polymers theaflavins of tea catechins have shown significant inhibitory function as well (Ferreira *et al.* 2009, Miyata *et al.* 2010, aus dem Siepen *et al.* 2015, Cappelli *et al.* 2018).

Preliminary data from ATTR CA patients who were administered EGCG daily for 1 year showed significant reductions in interventricular septal thickness and left ventricular myocardial mass, probably due to a reduction of myocardial amyloid burden.

Due to its different binding sites, a beneficial effect of the association between EGCG and tafamidis was suggested, with a potential synergistic effect on ATTR stabilization. However, its application may be restrained, as EGCG interacts with human serum albumin, affecting the bioavailability of tafamidis (Ferreira *et al.* 2009, Miyata *et al.* 2010, aus dem Siepen *et al.* 2015, Cappelli *et al.* 2018).

Catechin and epicatechin also seem to decrease oxidative stress on the endothelium by increasing nitric oxide release and bioavailability, thereby causing vasodilation. This represents an important feature, as endothelial dysfunction is one of the mechanisms in HF associated with a poor prognosis. Dark chocolate is also rich in flavonols, and it has been shown to decrease endothelial dysfunction and NT-proBNP levels when administered chronically in patients with HF, and was associated with a decrease in HF hospitalizations in small observational studies (Mostofsky *et al.* 2010, Dural *et al.* 2022).

Therefore, these substances represent a possible therapeutic strategy as they prevent the formation of amyloid fibrils and result in non-toxic or less harmful products, with high tolerability and low toxicity, although survival improvements have not been demonstrated (Ferreira *et al.* 2009, Miyata *et al.* 2010, aus dem Siepen *et al.* 2015, Cappelli *et al.* 2018).

Although inflammation and oxidative damage were proven to play a pivotal role in the development and progression of HFpEF, particularly in CA, research on targeting suppression and modulation of immune responses in this specific setting was not yet conclusive.

Other antioxidants

N-acetylcysteine, an ROS blocker, has thus so far only been tested in patients with acute myocardial infarction, with consistently inconclusive and conflicting results (Pasupathy *et al.* 2017). A study of free radical scavenger treatment (vitamin C, vitamin E, and acetylcysteine) in patients with hereditary transthyretin amyloidosis with polyneuropathy showed no decrease in hydroxynonenal – a product of lipid peroxidation. However, an increased nutritional status was recorded for liver-transplanted patients, suggesting scavenger treatment may facilitate recovery after transplantation (Suhr *et al.* 2001).

Chelating agents

As previously mentioned, transition metal ions are involved in oxidative damage and light chain-induced toxicity. The addition of metal chelators or metal-binding 8-hydroxyquinoline compounds (Chelex, PBT2, and clioquinol) was proven to be effective in blocking the ROS production and preventing the cardiotoxic effects of amyloid light chains in simple multicellular models (Diomede *et al.* 2017), laying the foundation for total inhibition of the vicious cycle of redox damage in humans.

Immunomodulatory and anti-inflammatory drugs

Several immunosuppressive and immunomodulatory drugs have been repurposed for the treatment of CA. Broad immunosuppression with corticosteroids, methotrexate, cyclosporin A, or intravenous immunoglobulins was hypothesized, mainly in the acute and ischemic setting, but without significant results (Ton *et al.* 2014, Michels da Silva *et al.* 2019). Studies involving TNF-α were held on patients with HF. Small studies investigated etanercept and infliximab, two TNF-α receptor antagonists, registering positive effects such as improved LVEF, 6MWT, and NYHA class. However, recent trials (i.e. RECOVER, RENAISSANCE, and ATTACH trials) showed no clinical benefit in chronic HF with NYHA III and IV (Mostofsky *et al.* 2010, Pasupathy *et al.* 2017, Dural *et al.* 2022). Studies on the inhibition of effector T and B lymphocytes are limited.

Soluble guanylyl cyclase (sGC), an enzyme activated by NO and involved in the cGMP-PKG signaling pathway, was recently shown to have beneficial effects on cardiac remodeling, thus becoming a target for therapy in HFpEF. Vericiguat, a sGC stimulator, was administered to patients with HFpEF, showing improvements in quality of life (Armstrong *et al.* 2020, McDonagh *et al.* 2021).

Cardiac contraction modulation therapy

In this setting, cardiac contraction modulation therapy (CCM) may play a role. It is an experimental HF device

therapy that delivers high-amplitude non-excitatory biphasic electrical signals to the myocardium during its refractory period. Eligible patients are individuals with NYHA class II or III, LVEF <50%, peak VO2 ≥10 mL/kg/min, and without arrhythmias (<PVCs 10,000/day) (McDonagh *et al.* 2021). It appeared to have a modulating effect on gene expression thanks to DNA interactions via specific electromagnetic response elements (EMREs), with a positive effect on fibrogenesis and inflammatory stress (Butter *et al.* 2008). CCM reverses cardiac maladaptive gene modifications and regulates some important sarcoplasmic reticulum and stretch-response genes such as A- and B-type natriuretic peptides, α-MHC, SERCA-2a, phospholamban, and ryanodine receptors. Moreover, it inhibits the TGF-β1 pathway, with reduced collagen production and fibroblast differentiation, therefore attenuating interstitial fibrosis (Zhang *et al.* 2016). The normalization of diastolic Ca2+ levels might lead to reductions of ROS and activation of CaMKII (Tschöpe *et al.* 2019). The p38 mitogen-activated protein kinase (p38MAPK) pathway is also downregulated. Lastly, CCM seems to facilitate the production of chaperone proteins, promoting the balance of protein synthesis and degradation and protecting against cell death in response to amyloid fibrils (Marchese *et al.* 2023).

Conclusion

The role of oxidative stress in the development and perpetuation of HF in CA has been extensively studied in recent years, and the consistent and sustained overproduction of ROS in such diseased cardiac tissue, largely overwhelming the antioxidant defense system, has been outlined as a primary pathogenic factor. In both light chain immunoglobulin and transthyretin amyloidosis, two major sources of ROS overproduction in the cardiomyocytes have been recognized: the upregulation of the membrane-bound enzyme NADPH oxidase (NOX) and the altered membrane potential of mitochondria. The pro-inflammatory effect of oxidative stress and damage further exacerbates the underlying inflammatory status, thus creating a vicious cycle that amplifies overall cardiac tissue damage.

Therefore, an adequate therapeutic approach that would suitably consider such a vicious cycle implying growing oxidative- and inflammatory-dependent heart damage in AL and ATRR amyloidosis looks really advisable. Although preclinical studies showed promising results by adopting various agents able to quench oxidative stress and damage, a significant impact on morbidity and mortality of AL and ATTR amyloidosis was not yet shown. Therefore, new clinical trials should evaluate therapeutic protocols that would more efficiently challenge the vicious cycle of oxidative damage and inflammation well evident in CA. One impelling reason for this is the fact that numerous patients with wtATTR amyloidosis, in particular, are elderly, an age group that shows a continuous percentage increase.

Declaration of interest

The authors declare no actual or potential conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

MB conceived and supervised the overall work; EC and AHM wrote the draft manuscript; and all authors reviewed the draft and approved the final version of the manuscript.

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