Targeting Amyloid Fibrils by Passive Immunotherapy in Systemic Amyloidosis

Mario Nuvolone1,2 · Alice Nevone1,2 · Giampaolo Merlini1,2

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Abstract
Systemic amyloidoses are characterized by the unrelenting deposition of autologous proteins as highly ordered fibrils in target organs. The ensuing, potentially fatal organ dysfunction is the result of the combined damage caused by the proteotoxic effect of prefibrillar species and by the cytotoxicity and the structural alterations produced by the amyloid fibrils. Current therapy is focused on eliminating the amyloid protein, thus extinguishing the amyloid cascade at its origin. While this approach may end the cell damage caused by prefibrillar aggregates and prevent further amyloid accumulation, the noxious effects of the amyloid fibrils persist and may hamper the recovery of organ function, which is the ultimate goal of therapy as it is necessary to improve the quality of life and extend survival. Preclinical studies indicate that the clearance of amyloid deposits can be accelerated by specific antibodies targeting amyloid fibrils that activate complement-mediated macrophages and giant cell phagocytosis, possibly promoting the recovery of organ function. Measuring the therapeutic effect of anti-amyloid agents is still a matter of research. In recent years, several monoclonal antibodies targeting amyloid deposits have been tested in clinical trials with mixed outcomes. Recent encouraging results from phase I/II trials, new anti-amyloid agents, and new antibody engineering offer hope that effective amyloid removal will be accomplished in the near future, accelerating organ recovery and improving quality of life and survival.

1 Introduction
Systemic amyloidoses are characterized by the unrelenting deposition of autologous proteins as highly ordered fibrils in target organs, determining their dysfunction [1, 2]. The mechanisms of organ damage caused by the amyloid process remain elusive despite intense investigations. In immunoglobulin light chain (AL) amyloidosis, there is evidence that organ dysfunction derives from the combined damage caused by the proteotoxic effect of the monoclonal immunoglobulin light chain (LC) prefibrillar species [3–5] and by the cytotoxicity and structural alterations produced by the amyloid fibrils [6, 7].

Key Points
While the most effective therapeutic strategy in systemic amyloidosis remains the lowering of the amyloidogenic precursors, which leads to reduced proteotoxicity and to the slow reabsorption of amyloid deposits, specific antibodies targeting amyloid deposits may accelerate amyloid clearance, possibly favoring the recovery of organ function resulting in improved patients’ quality of life and extended survival.

Several monoclonal antibodies targeting different components of the amyloid deposits have been tested in different clinical settings with mixed outcomes, possibly related to the antibody specificity and accessibility to amyloid deposits in the heart.

Further research for new workable amyloid fibril targets and validated criteria to assess the organ response to anti-amyloid therapies are needed. Recent outcomes from phase I studies offer hope that effective amyloid removal may be accomplished.
In transthyretin (ATTR) amyloidosis, the proteotoxicity of prefibrillar aggregates [8, 9] is probably less prominent than in AL amyloidosis, while the architectural distortion produced by amyloid deposits may play a main role, as suggested by magnetic resonance imaging (MRI) investigation of the extracellular volume in the heart [10]. Extracellular myocardial ATTR deposits accumulate in massive amounts, impacting such mechanical properties of the myocardium as diastolic relaxation, followed by systolic impairments during progression of the disease.

The current therapy is focused on eliminating the amyloid protein in AL amyloidosis through chemotherapy and immunotherapy against the underlying B-cell/plasma-cell clone [11–13], and in ATTR amyloidosis by the suppression of the synthesis of the amyloid precursor with gene silencers/editing, while TTR tetramer kinetic stabilizers reduce the formation of amyloidogenic misfolded monomers, thus extinguishing the amyloid cascade at its origin [14, 15]. This approach may end the cell damage caused by prefibrillar aggregates and prevent further amyloid accumulation; however, the noxious effects of the amyloid fibrils persist and may hamper the recovery of organ function, which is the goal of therapy. The importance of amyloid fibril removal is particularly relevant for patients with more advanced diseases, where strategies aimed at reducing the availability of the misfolded protein precursor cannot address existing organ amyloid burden and dysfunction. Investigators have focused their efforts on promoting the reabsorption of amyloid deposits, mainly through passive immunotherapy and, to a lesser extent, through fibril disruption by small molecules, such as doxycycline and epigallocatechin-3-gallate [16, 17]. This review focuses on the attempts to remove amyloid deposits through anti-fibril monoclonal antibodies.

2 Recovery of Organ Function

To improve the quality of life and extend survival in systemic amyloidoses, it is necessary to halt the end-organ damage to vital organs and possibly restore their function. In AL amyloidosis, the severity of cardiac involvement is the determinant of survival. It is possible to grade the degree of damage in three stages using a marker of cardiomyocyte damage, troponins (cTn), and a marker of cardiomyocyte dysfunction, the amino-terminal portion of the pro-natriuretic peptide type B (NT-proBNP) [18]. A large European study showed that patients with extremely high values of NT-proBNP (> 8500 ng/L, stage IIIb) have severe heart failure and a dramatically short survival of only 4 months [19]. Pathology studies have shown that the process of amyloid deposition is associated with loss of cardiomyocytes [20], probably caused by the cytotoxicity exerted by the prefibrillar and fibrillar aggregates. These findings strongly support the necessity to start treatment in the early stage of the disease, to be able to fully recover the organ function by a combined action on the synthesis of the amyloid protein precursor and the amyloid deposits.

However, there is hope that even in patients with advanced cardiac damage (stage IIIa and IIIb), the removal of the amyloid deposits may improve the mechanical action of the heart. This hypothesis is presently tested in two phase III trials in AL amyloidosis, combining anti-clone therapy with anti-fibril therapy (NCT04512235, NCT04504825).

3 Monitoring the Therapeutic Effect

Although response criteria to anti-clone therapy have been established and validated [21], there are no validated criteria to evaluate the response to anti-amyloid fibril therapies, and this is reflected by the heterogeneous endpoints of the trials testing antibodies targeting amyloid deposits. In particular, the lack of validated methods to reliably evaluate the burden of amyloid deposits in the heart has promoted the use of clinical/functional endpoints with criteria that were not validated in systemic amyloidosis. Since the improvement of cardiac function is the most relevant endpoint, criteria for the reliable assessment of the cardiac response to anti-amyloid therapy are needed. In 2020, the Amyloidosis Forum launched an initiative to identify novel/composite endpoints and analytic strategies to expedite clinical trials for the development of new therapies. Working groups comprised of amyloidosis experts, patient representatives, statisticians, representatives from the US FDA, the UK Medicines and Healthcare Products Regulatory Agency, and pharmaceutical companies produced recommendations for different organs. The prioritized cardiac endpoints relevant to AL amyloidosis in the context of an anti-clone therapy have been recently published [22]. Although the endpoints were not developed specifically for anti-amyloid therapies, they may offer clues in this context. The endpoints included overall survival, hospitalization, NT-proBNP level, 6-min walking test (6MWT), Kansas City Cardiac Questionnaire, and cardiac deterioration progression-free survival. The Working Group also supported further investigation of imaging in drug development programs, and the outcome of these studies will establish the role of imaging in monitoring the response to anti-amyloid therapies.

The International Society of Amyloidosis guidelines for therapy and monitoring of ATTRv amyloidosis report the criteria for the evaluation of cardiac and neurologic response, which may provide guidance for endpoints to be used in anti-amyloid trials [23]. The authors propose
a common minimum set of evaluations to monitor the course of the polyneuropathy in response to therapy, which includes an evaluation of the polyneuropathy disability (PND) score, the 6-min or 10-m walk test depending on the severity of the neuropathy, the composite clinical neuropathy impairment score (NIS) by examination, and ability in daily life, by the Rash-built overall disability scale [23]. Assessing the response to anti-amyloid therapy in ATTR cardiac amyloidosis is also problematic due to the lack of validated criteria [23, 24]. Assessment of cardiac disease status should be part of a multiparametric evaluation. A consensus panel provided criteria of disease progression during therapy belonging to three categories [25]: (1) clinical and functional endpoints (any hospitalization for heart failure; any increase in New York Heart Association [NYHA] class; 10% decline in EQ-5D score; 40 m decrease in 6MWT); (2) biomarkers and laboratory markers (30% increase in NT-proBNP with 300 ng/L cut-off; 30% increase in high-sensitivity troponin; and (3) imaging and electrocardiographic parameters (left ventricle ejection fraction decrease > 5%; > 5 mL decrease in SV; ≥ 2 mm increase in left ventricle wall thickness; new-onset atrial fibrillation or atioventricular block or bundle branch block; stepwise increase in diastolic dysfunction grade). The authors suggested using one marker from each category to assess response to therapy. Further research is necessary to validate criteria to assess the organ response to anti-amyloid therapies and establish surrogate biomarkers to expedite trial conduction. To achieve these goals, international collaboration, including pharma industries, regulatory agencies, and patient organizations, is necessary.

4 Anti-Amyloid Passive Immunotherapy

Although there are approximately 20 proteins causing systemic amyloidosis, in 95% of patients the disease is caused either by LC or TTR. Efforts to develop anti-amyloid antibodies have been directed toward these two proteins, in addition to pan-amyloid approaches.

The amyloid heart involvement is the determinant of survival in AL and ATTRwt amyloidoses and the main determinant in patients with ATTRv amyloidosis with mixed heart and nerve phenotypes. Removing amyloid deposits from the heart is the main target of passive immunotherapy. One of the critical factors in developing effective passive immunotherapy is the choice of target (epitope). The design of the anti-amyloid antibodies was different for each antibody tested in the clinic (Table 1). All therapeutic monoclonal antibodies used in trials in systemic amyloidosis, either anti-plasma cells or anti-fibrils, are immunoglobulin (Ig) G1 due to the capability of this subclass to activate complement, mediate complement-dependent cytotoxicity, and recruit effector cells for antibody-dependent cellular cytotoxicity against human target cells. Furthermore, this subclass binds to the human neonatal Fc receptor (FcRn) that protects IgG1 molecules from degradation and thereby extends their serum half-life. Additionally, human IgG1 antibodies are stable, form fewer aggregates, and have favorable biotechnological characteristics that facilitate their production and cost containment [26].

4.1 Targeting Common Constituents of Amyloid Deposits

4.1.1 Dezamizumab and Miridesap

Pepys and collaborators pursued the brilliant project to develop an antibody capable of recognizing and promoting the resorption of amyloid deposits regardless of the protein precursor, exploiting the presence of the normal plasma protein serum amyloid P component (SAP) as a common constituent of all amyloid deposits [27]. The therapy included the depletion of SAP from plasma by the drug (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC, miridesap). This drug is a competitive inhibitor of SAP binding to amyloid fibrils and also crosslinks and dimerizes SAP molecules, leading to their very rapid clearance by the liver, and thus produces a marked depletion of circulating human SAP [28]. The safety, tolerability, pharmacokinetics, and pharmacodynamics of miridesap alone have been investigated in both healthy volunteers and patients with systemic amyloidosis (NCT01323985, NCT02953808, NCT01406314), with a lack of treatment-related adverse events or safety signals and a dose-dependent depletion of circulating SAP up to > 95% of normal levels [29, 30].

CPHPC leaves some SAP in amyloid deposits that can be specifically targeted by therapeutic IgG anti-SAP antibodies. The activation of the classic complement pathway by the monoclonal IgG1 anti-SAP-antibodies leads to the opsonization of amyloid deposit and attracts a massive number of macrophages that fuse into multinucleated giant cells that reabsorb amyloid deposit, as observed in an animal model of amyloid A type amyloidosis [31].

The humanized IgG1 anti-SAP antibody (dezamizumab) in combination with miridesap has been tested in an open-label, single dose-escalation, phase I trial (NCT01777243) (Table 2), which aimed to evaluate the safety and pharmacokinetic profile of this combination therapy [32]. Patients with different types of systemic amyloidosis and with evidence of splenic and/or hepatic amyloid involvement were eligible to enter this study, while subjects with cardiac amyloid involvement were not included for safety reasons. The first part of the trial was a single-center, open-label, single dose-escalation study testing intravenous administration of dezamizumab (from 0.1 to 30 mg/kg) combined with miridesap. Results on 15 treated patients (8 AL, 4 AFib,
Table 1: Characteristics of monoclonal antibodies targeting amyloid deposits used in interventional clinical trials

| Type of antibody | Ig subclass | Target | Affinity | Half-life | References |
|------------------|-------------|--------|----------|-----------|------------|
| Dezamizumab (dezamizumab) | Human IgG1 | SAP | $K_D = 1.5\text{ nM}$ (dezamizumab) | 4–16 h | [31, 32] |
| Birtamimab (NEOD001) | Human IgG1 | Conformational neoepitope contained within the first 18 amino acids of misfolded human LC | $EC_{50} = 10\text{ nM}$ (synthetic AL amyloid fibrils) | 10–16 days | [44, 45, 47] |
| Anselamimab (CAEL-101) | Human IgG1 | The cryptotope peptide TTR89–97 exposed to solvent in misfolded TTR species | $K_D$ of the four murine moAbs for monomeric misfolded TTR variants F87M/H110M ranged from 18.2 to 7.68 nM | 10–16 days | [54, 55, 57, 53] |
| PRX004 | Human IgG1 | Wild-type ATTR amyloidosis | $K_D$ of the four murine moAbs reported by Higaki et al. [60] | Not reported | [59] |

**References**

[31, 32, 33, 34, 44, 45, 47, 54, 55, 57, 53, 60, 61]
| Antibody                        | Study design                  | Setting                              | Purpose               | Key eligibility criteria                                                                 | Key exclusion criteria                                                                 | References |
|--------------------------------|-------------------------------|--------------------------------------|-----------------------|------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|------------|
| Dezamizumab (GSK2398852)       | Uncontrolled, open-label,     | Different types of systemic amyloidosis | Safety and PK profile | Systemic amyloidosis with spleen ± liver involvement                                      | Cardiac amyloid involvement at echocardiography; decompensated cardiac failure or a recent history of syncope associated with cardiac disease; clinically significant ECG abnormalities; eGFR < 30 mL/min; active vasculitis; dementia or CAA | [32]       |
|                                | single-center, single DE     |                                       |                       |                                                                                          |                                                                                         |            |
| + miridesap (GSK2315698)       |                               |                                       |                       |                                                                                          |                                                                                         |            |
| NCT01777243                    | Phase I (Part A)              |                                       |                       |                                                                                          |                                                                                         |            |
| Dezamizumab (GSK2398852)       | Uncontrolled, open-label,     | Different types of systemic amyloidosis | Dose-response         | Systemic amyloidosis with spleen ± liver involvement                                      | eGFR < 30 mL/min; active vasculitis; dementia or CAA                                    | [33]       |
|                                | two-center                    |                                       |                       |                                                                                          |                                                                                         |            |
| + miridesap (GSK2315698)       |                               |                                       |                       |                                                                                          |                                                                                         |            |
| NCT01777243                    | Phase I (Part B)              |                                       |                       |                                                                                          |                                                                                         |            |
| Dezamizumab (GSK2398852)       | Uncontrolled, open-label      | Group 1: Cardiac ATTR-wt/v            | Safety and efficacy   | Group 1: LGE on CMR indicative of cardiac amyloidosis and LV mass > 200 g; clinically stable NYHA II–III | Unrelated cardiomyopathy; NT-proBNP > 8500 ng/L; eGFR < 40 mL/min; ALT > 3 × ULN and bilirubin > 1.5 × ULN; symptomatic autonomic neuropathy; hypoalbuminemia | [34]       |
| + miridesap (GSK2315698)       |                               |                                       |                       |                                                                                          |                                                                                         |            |
| NCT03044353                    |                               |                                       |                       |                                                                                          |                                                                                         | [32]       |
|                                | Phase II                      | Group 2: AL post-therapy              |                       | Group 2: AL with ≥ VGPR to therapy terminated ≥ 6 months and not requiring chemotherapy    | For AL patients: NYHA III/IV; decompensated cardiac failure or a recent history of syncope/ presyncope; NT-proBNP > 1800 ng/L |            |
|                                |                               | Group 3: Newly diagnosed AL with ongoing chemotherapy |                       |                                                                                          |                                                                                         | [47]       |
|                                |                               |                                       |                       |                                                                                          |                                                                                         |            |
| Birtamimab (NEOD001)           | Uncontrolled, open-label,     | AL patients post-therapy, persistent organ dysfunction | DE: MTD and RP2D - Expansion: safety, preliminary efficacy, PK   | AL with ongoing organ dysfunction; ECOG 0–2; one or more lines of anti-plasma cell therapy with PR or better | NT-proBNP > 5000 ng/L; life expectancy < 3 months; symptomatic MM; patients requiring plasma cell-directed therapy | [47]       |
|                                | multi-site, DE                |                                       |                       |                                                                                          |                                                                                         |            |
| NCT01707264                    | Phase I/II                    |                                       |                       |                                                                                          |                                                                                         |            |
### Table 2 (continued)

| Antibody | Trial number | Phase | Study design | Setting | Purpose | Key eligibility criteria | Key exclusion criteria | References |
|----------|--------------|-------|--------------|---------|---------|--------------------------|------------------------|------------|
| Birtamimab (NEOD001) NCT02632786 | Phase IIb | Global, multicenter, randomized, double-blind, placebo-controlled, two-arm, parallel-group | AL patients post-therapy, persistent cardiac dysfunction | Efficacy and safety | AL with cardiac involvement; NT-proBNP ≥650 ng/L; one or more prior systemic plasma cell dyscrasia therapies with ≥PR | NT-proBNP > 5000 ng/L; concomitant MM; patients receiving plasma cell-directed therapy within 6 months; patients receiving ASCT within 12 months | [48] |
| Birtamimab (NEOD001) NCT02312206 | Phase III | Global, multicenter, randomized, double-blind, placebo-controlled, two-arm | Newly diagnosed AL patients receiving Bz-based therapy | Efficacy and safety | Newly diagnosed, therapy-naive AL; cardiac involvement; planned first-line chemotherapy contains a PI agent administered weekly | Symptomatic MM; eligible for and plans to undergo ASCT | [49] |
| Anselamimab (CAEL-101) NCT02245867 | Phase Ia/b | Uncontrolled, open-label, single-center, 'up-and-down’ DE | AL not presently requiring life-prolonging or life-saving therapy | Tolerance, safety, PK, possible clinical benefit | Measurable, localized AL, or systemic AL; RR to systemic therapy or declined or not eligible for life-prolonging or life-saving systemic therapy | Renal failure (on dialysis); ECOG > 3; seriously limited cardiac, renal, or hepatic function | [57] |
| PRX004 NCT03336580 | Phase I | Uncontrolled, open-label, DE | ATTRv | Safety, tolerability, PK, PD, MTD | ATTRv; if cardiac amyloid, NT-proBNP 650–5000 ng/L or IVS > 1.2 cm; PND ≤ IIIb, NIS: 5–130; if treatment with tafamid or diflunisal, stable dosing in the previous 6 months | Prior or planned liver transplant; NYHA > II, EF ≤45%; mBMI ≤ 600 kg/m² × g/L; uncontrolled symptomatic orthostatic hypotension; recent treatment with patisiran or inotersen | [61] |

**AL** AL amyloidosis, **ALT** alanine transaminase, **ASCT** autologous stem cell transplant, **ATTRv** variant ATTR amyloidosis, **ATTRwt** wild-type ATTR amyloidosis, **Bz** bortezomib, **CAA** cerebral amyloid angiopathy, **CMR** cardiac magnetic resonance, **CR** complete response, **DE** dose escalation, **ECG** electrocardiogram, **ECOG** Eastern Cooperative Oncology Group, **EF** ejection fraction, **eGFR** estimated glomerular filtration rate, **FLC** free light chain, **IVS** interventricular septum, **LGE** late gadolinium enhancement, **LV** left ventricle, **mBMI** modified body mass index, **MM** multiple myeloma, **MTD** maximum tolerated dose, **NIS** neuropathy impairment score, **NT-proBNP** amino-terminal pro-brain natriuretic peptide, **NYHA** New York Heart Association classification of heart failure, **PD** pharmacodynamics, **PK** pharmacokinetic, **PND** polyneuropathy disability score, **PI** proteasome inhibiting, **PR** partial response, **RP2D** recommended phase II dose, **RR** relapsed/refractory, **ULN** upper limit of normal, **VGPR** very good partial response.
mild to moderate urticaria or macular rashes were seen in most recipients of dezamizumab ≥ 600 mg. Two skin biopsies were performed, one showing dermal leukocytoclastic vasculitis (LCV) and the other demonstrating only non-specific changes. Pharmacokinetic studies confirmed the observations from the first part of the trial, with dezamizumab concentration rapidly decreasing, particularly in patients with heavy amyloid load and C3 depletion. A decrease in liver stiffness, reduced extracellular volume, and reduced hepatic, renal, splenic, or adrenal amyloid load by 123I-SAP-scintigraphy were documented in a subset of cases, particularly after infusion with the highest doses of dezamizumab. Amyloid clearance from the liver occurred faster and was more conspicuous compared with other organs. Among the six patients with cardiac amyloidosis (three AL and three ATTR), in four cases a transient, up to fivefold increase in serum NT-proBNP concentration was noted, which lasted for about 1 week and was taken to suggest the engagement of the target mechanism in the heart. However, no systematic change in left ventricular mass by cardiac MRI, or change in cardiac uptake at 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scintigraphy (for the three ATTR cases) was documented [33].

Based on these results, a larger study with serial anti-SAP dosing at regular and closer intervals was designed to extend safety and establish the efficacy of miridesap/dezamizumab combination therapy in cardiac amyloidosis (NCT03044353) (Table 2). This was a single-arm, open-label, phase II study evaluating monthly repeat anti-SAP treatment in systemic amyloidosis participants with cardiac dysfunction caused by cardiac amyloidosis. The study was planned to include three groups of patients [34]. Group 1 consisted of patients with cardiac ATTR (either ATTRwt or ATTRv); group 2 included patients with cardiac AL amyloidosis having already achieved at least a very good partial response to previous chemotherapy and not requiring further anti-plasma cell therapy at study initiation; and group 3 was deemed to include newly diagnosed, cardiac AL patients having attained a free LC complete response after three cycles of chemotherapy, with at least the first cycle consisting of a combination of cyclophosphamide, bortezomib, and dexamethasone. Patients with NT-proBNP levels exceeding 8500 ng/L who had a very poor median survival of 4 months [19] were excluded from the trial. The experimental treatment consisted of an initial infusion of miridesap on days −2 and −1 (which could eventually be repeated once daily up to day 3) to achieve a serum concentration of SAP below 3 mg/L, followed by dezamizumab infusion (600–1200 mg, split into two doses at day 1 and day 3), accompanied by up to three-times-daily subcutaneous injections of miridesap from day 1 to day 11 (Table 3). Six dezamizumab treatments per patient were planned, with 1-month intervals between each session. Six patients from group 1 with cardiac ATTR amyloidosis received between three and six treatment sessions (total dezamizumab dose between 3000 and 6000 mg). The presence of anti-dezamizumab antibodies could be demonstrated in all patients receiving four or more treatment sessions at the 8-week follow-up and persisted at 6-month follow-up visits. This high rate of antidrug antibody formation is higher than expected for a humanized antibody [35]. The isotype of these anti-dezamizumab antibodies and their potential impact on dezamizumab bioavailability, pharmacokinetic/pharmacodynamic properties, efficacy, and safety was not reported [35–37].

Treatment-related adverse events occurred in all but one of the seven enrolled patients, the most frequent consisting of an urticarial and/or maculopapular rash, with histopathological evidence of LCV in both cases where a skin biopsy was performed. Notably, in the first AL patient enrolled in group 2, following initial dezamizumab dosing (300 mg), a treatment-related serious adverse event occurred, consisting of a presumptive abdominal large-vessel vasculitis (aortitis) (Table 3) [34]. It is of interest that SAP is also a normal component of several basement membranes, including the glomerular, alveolar, and some vascular basement membranes [38–40]. The potential binding of the mAb to SAP in vessels is supposed to be the basis of dermal LCV, manifesting as a rash. There is the potential for overlap between dermatological manifestations of LCV and an event of large-vessel vasculitis [34]. Regarding efficacy data, no consistent change in left ventricular mass, left ventricle wall thickness or extracellular volume at cardiac MRI could be documented. Furthermore, there was no consistent pattern of change in cardiac functional measures or NT-proBNP levels during the study period [34].

A concurrent immune positron emission tomography (PET) study on patients with cardiac amyloidosis (NCT03417830) showed only moderate to low cardiac uptake of 89Zr-dezamizumab on the first two cardiac ATTR amyloidosis patients enrolled [34]. In addition to impaired cardiac dezamizumab microvascular perfusion, this may also be a result of the continuous structure of the endothelium in cardiac tissue, in contrast to the fenestrated endothelium found in the liver and spleen, the organs in which the most striking treatment effects had been previously reported [32]. The concentration of dezamizumab in cardiac tissue may have been insufficient to trigger macrophage and giant cell recruitment and amyloid clearance. This observation raises the general critical question of the therapeutic antibodies' access to cardiac amyloid deposits. Occurrence of the treatment-related serious adverse event in conjunction with the lack of cardiac signal of efficacy led to a reconsideration of the benefit–risk assessment for the treatment, and both clinical trials and the development of this treatment for cardiac amyloidosis were terminated [34].

△ Adis
| Antibody                  | Trial number Phase | Trial status | Enrolled patients reported | Primary and secondary endpoint | Treatment | Treatment duration | Results                                                                 | AE/SAE                                                                 | References |
|--------------------------|--------------------|--------------|----------------------------|--------------------------------|-----------|-------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------|------------|
| Dezamizumab (GSK2398852) | NCT01777243        | Completed    | 15 patients (8 AL, 4 AFib, 2 AA, 1 AApoAI) | **Primary:** Safety and PK **Secondary:** SAP concentration measurements; anti-drug Ab | Dezamizumab 0.1, 1, 3, 10, 30 mg/kg IV + miridesap (variable administration) | Single dose (follow-up data up to 45 days) | Rapid depletion of circulating SAP and rapid disappearance of dezamizumab from the circulation, consistent with segregation in amyloid; decrease in liver stiffness, reduced extracellular volume, and reduced hepatic/renal/lymph nodal amyloid load by SAP scintigraphy in a subset of cases | No SAE reported; brief acute-phase response and self-limiting symptoms in patients receiving >200 mg of dezamizumab | [32]       |
| Dezamizumab (GSK2398852) | NCT01777243        | Completed    | 23 patients (12 AL, 5 AFib, 3 ATTR, 2 AA, 1 AApoAI) | **Primary:** Dose-response to treatment **Secondary:** SAP concentration measurements; anti-drug Ab | Dezamizumab up to 2000 mg + miridesap (variable administration) | Up to three treatments with at least a 2-month interval | Decrease in liver stiffness, reduced extracellular volume, and reduced hepatic/renal/spleen/adrenal amyloid load by SAP scintigraphy in a subset of cases Transient NT-proBNP increase, no change in LVM by CMR, and no change in cardiac uptake at DPD scintigraphy in most patients | SAE: hypotension, tachycardia ↑sCreat (1 patient); erythema multiforme-like rash (1 patient); AF (1 patient) Infusion reactions: urticarial or macular rashes | [33]       |
| Antibody                  | Trial number Phase                                      | Trial status                      | Enrolled patients reported | Primary and secondary endpoint                                                                 | Treatment                                                                 | Treatment duration | Results                                                                 | AE/SAE                                                                 | References         |
|--------------------------|--------------------------------------------------------|----------------------------------|-----------------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------|
| Dezamizumab (GSK2398852) + miridesap (GSK2315698) NCT03044353 Phase II | Terminated (change in benefit/risk profile)            | 7 patients (6 in Group 1; 1 in Group 2) | Primary: Change in LVM at CMR over time from baseline; AE/SAE Second: Additional echo/CMR data; PK/PD data; additional safety data | Dezamizumab (600–1200 mg) split into two infusions on days 1 and 3 + miridesap (variable administration) | Up to six treatment sessions | No consistent change in LVM, LV thickness, or ECV at CMR/ echocardiography No consistent pattern of change in cardiac functional measures or NT-proBNP levels Anti-drug Ab in all patients receiving four or more treatment sessions | Anti-drug Ab in all patients receiving four or more treatment sessions | Presumptive abdominal large-vessel vasculitis (1 patient) Urticarial or macular rash, LCV | [34] |
| Birtamimab (NEOD001) NCT01707264 Phase I/II | Completed DE: 27 patients Total: 69 patients | DE: 27 patients Total: 69 patients | Primary: MTD; RP2D; safety; tolerability Second: PK; anti-drug Ab; organ response (expl.) | DE (3+3): 0.5, 1, 2, 4, 8, 16, 24 mg/kg IV every 28 days | ≤1 year | MTD not achieved; no DLTs, anti-drug Ab or hypersensitivity reactions; all patients escalated to 24 mg/ kg; cardiac and renal responses in 57% and 60% of evaluable patients, respectively; half-life of NEOD001: 13–16 days | AE grade 3 or higher: Hypotremia (7%); diverticulitis (4%); pneumonia (4%) | [47] |
| Antibody          | Trial number       | Trial status            | Enrolled patients reported | Primary and secondary endpoint | Treatment                  | Treatment duration | Results                                      | AE/SAE | References       |
|-------------------|--------------------|-------------------------|----------------------------|--------------------------------|---------------------------|-------------------|---------------------------------------------|--------|-----------------|
|                   |                    |                         |                            |                                |                           |                   |                                             |        |                 |
| Birtamimab (NEOD001) | NCT02632786        | Completed               | 129 patients (66 drug; 63 placebo) | Primary: Cardiac response  <br>Secondary: Renal/hepatic response; QoL; functional measures | NEOD001: 24 mg/kg IV every 28 days <br>Placebo: 250 mL saline IV every 28 days | ≤1 year          | Cardiac response (ITT analysis) in 26/66 (39.4%) patients in the NEOD001 arm and 30/63 (47.6%) patients in the placebo arm (p = 0.319)  <br>Renal response in 7/13 (53.8%) patients in the NEOD001 arm and 6/18 (33.3%) patients in the placebo arm (p = 0.3529)  <br>Liver response in 1/5 (20%) patients in the NEOD001 arm and 0/4 (0%) patients in the placebo arm (p = 0.4142) | SAE in 14/66 (21.2%) patients in the NEOD001 arm and 15/63 (23.8%) patients in the placebo arm | [48] |
|                   |                    |                         |                            |                                |                           |                   |                                             |        |                 |
| Birtamimab (NEOD001) | NCT02312206        | Terminated (futility analysis) | 260 patients (130 drug; 130 placebo) | Primary: Time to composite of all-cause mortality or cardiac hosp.  <br>Secondary: Organ response; functional measures | NEOD001: 24 mg/kg IV every 28 days <br>Placebo: 250 mL saline IV every 28 days | Estimated study duration of 42 months (event-driven trial) | Death or cardiac hosp.: 56/130 (43.1%) of patients in the NEOD001 arm and 62/130 patients (47.7%) in the placebo arm  <br>Time to death or cardiac hosp. (ITT analysis): Log-rank test: p = 0.3300; HR 0.835 (two-sided 95% CI 0.5799–1.2011) | SAE in 88/130 (67.7%) patients in the NEOD001 arm and 91/130 (70%) patients in the placebo arm | [49] |
| Antibody        | Trial status | Enrolled patients reported | Primary and secondary endpoint | Treatment | Treatment duration | Results | AE/SAE                                                                 | References |
|-----------------|--------------|----------------------------|-------------------------------|-----------|-------------------|---------|------------------------------------------------------------------------|------------|
| Anselamimab (CAEL-101 NCT02245867 Phase Ia/b) | Completed    | 27 patients                | Primary: MTD; safety<br>Secondary: PK; organ response | DE: 0.5, 5, 10, 50, 100, 250, 500 mg/m² until MTD, expansion of the two highest dose levels | Phase Ia: single dose; Phase Ib: four weekly injections | No DLT up to 500 mg/m²; MTD not achieved in either phase Ia or Ib; half-life of CAEL-101: 10–16 days; cardiac and renal responses in 67% and 20% of evaluable patients, respectively | AE grade 3 or higher: pruritus (12.5%), pericardial effusion (5.3%) | [57] |
| PRX004 NCT03336580 Phase I | Terminated (COVID-19) | DE: 21 patients<br>Long-term extension: 17 patients | Primary: MTD; treatment-emergent AEs<br>Secondary: PK; anti-drug Ab | DE: 0.1, 0.3, 1, 3, 10, 30 mg/kg IV every 28 days<br>Expansion/long-term extension at RP2D | DE: up to 3 infusions<br>Long-term extension: up to 15 infusions | PK consistent with IgG1 monoclonal antibodies; pooled data from cohorts >3 mg/kg on 7 evaluable patients; improved GLS in 7 patients and improved NIS in 3 patients | No drug-related SAE; AEs (>10%): fall, anemia, URTI, back pain, constipation, diarrhea, insomnia | [61] |

**Notes:**
- AA amyloid A amyloidosis, Ab antibodies, AE adverse event, AF atrial fibrillation, AFib fibrinogen Aα-chain amyloidosis, AL AL amyloidosis, AAPoAI apolipoprotein A-I amyloidosis, ATTR transthyretin amyloidosis, CMR cardiac magnetic resonance, CI confidence interval, COVID-19 coronavirus disease 2019, DE dose escalation, DLT dose-limiting toxicity, DPD 3,3-diphospho-1,2-propanodicarboxylic acid, echo echocardiogram, ECV extracellular volume, expl. exploratory, GLS global longitudinal strain, hosp. hospitalization, HR hazard ratio, Ig immunoglobulin, ITT intention-to-treat, IV intravenous, LCV leukocytoclastic vasculitis, LV left ventricle, LVM left ventricle mass, MTD maximum tolerated dose, NIS neuropathy impairment score, NT-proBNP amino-terminal pro-brain natriuretic peptide, PD pharmacodynamic, PK pharmacokinetic, QoL quality of life, RP2D recommended phase II dose, SAE serious adverse event, SAP serum amyloid P component, sCreat serum creatinine level, URTI upper respiratory tract infection, ↑ indicates increased
4.1.2 AT-01, AT-02, AT-03

Similar pan-amyloid approaches, although not based on targeting amyloid deposits with antibodies, have been developed by Wall and collaborators. A radiolabeled polybasic peptide (also known as AT-01) that binds via electrostatic interactions to electronegative glycosaminoglycans and protein fibrils, two common components of amyloid deposits, is being used for imaging systemic amyloidosis in AL and ATTR amyloidosis in a phase II study (NCT05235269) [41]. This peptide was fused with the Fc portion of IgG1 immunoglobulin (AT-02) to promote the phagocytic reabsorption of amyloid deposits [42]. The preclinical characterization of a novel fusion protein of SAP with IgG1 Fc portion (AT-03), with pan-amyloid binding and removal properties, showed that a single intravenous dose of AT-03 resulted in a significant reduction of mouse splenic AA amyloid within 14 days post-injection [43].

4.2 Targeting the Amyloid Deposits in AL Amyloidosis

4.2.1 Birtamimab

The murine monoclonal antibody 2A4, whose humanized form is birtamimab, binds an epitope derived from a cleavage site of serum amyloid protein A containing a -Glu-Asp- amino acid pairing [44]. The antibody also binds LC amyloid fibrils, both κ and λ. Plasmon resonance analyses using synthetic AL fibrils as a substrate revealed that 2A4 bound with a KD of 10 nM. Radiolabeled 2A4 specifically localized with human AL amyloid extracts implanted in mice (amyloidomas) as shown by single-photon emission computed tomography (SPECT) imaging. Treatment with 2A4 accelerates regression of AL κ amyloidomas in mice, compared with animals that received a control antibody [45].

A subsequent study reported that 2A4 bound both soluble and insoluble LC aggregates extracted from patient tissue, and induced macrophage engagement and phagocytic clearance of AL amyloid deposits in vitro [46].

Birtamimab (NEOD001) was tested in a first-in-human, uncontrolled, open-label, multicenter, phase I/II study on AL amyloidosis patients with persistent amyloid organ dysfunction despite having achieved partial hematologic response or better to at least one previous line of anti-plasma cell therapy (NCT01707264) (Table 2) [47]. This trial aimed first at establishing the maximum tolerated dose or phase II recommended dose of birtamimab when infused as a single agent in patients with AL amyloidosis, and then at evaluating birtamimab safety, preliminary efficacy, and pharmacokinetics in the same clinical setting. Patients with NT-proBNP levels above 5000 ng/L, a life expectancy < 3 months, the presence of a co-existing, symptomatic multiple myeloma, and patients requiring plasma cell-directed therapy were excluded from the trial. The dose-escalation phase of the study had a 3 + 3 design, with seven dose cohorts covering the range of 0.5–24 mg/kg, until the achievement of the maximum tolerated dose (defined as the highest dose level at which no more than one of six patients experienced dose-limiting toxicity during the first month of therapy). Birtamimab was administered every 28 days, with a treatment duration of up to 1 year. The interim results on the 27 patients enrolled in the dose-escalation phase of the study were initially reported [47]. There were no dose-limiting toxicities and hence the maximum tolerated dose was not achieved. All enrolled patients could be scaled up to 24 mg/kg of birtamimab, which was established as the phase II recommended dose (Table 3). Furthermore, there were no clinical manifestations attributable to hypersensitivity, and no anti-birtamimab antibodies could be detected. The terminal elimination half-life of birtamimab in serum was between 13 and 16 days (Table 1), with a pharmacokinetic profile consistent with dosing by intravenous infusion every 28 days across the dose range. This is in stark contrast with the very short half-life of dezamizumab that was attributed to the sequestration of this antibody by the amyloid deposits. The reason for this discrepancy between two antibodies that are both supposed to target amyloid deposits has not been clarified. Exploratory secondary endpoints also included correlation studies of birtamimab exposure levels with changes in biomarkers of amyloid organ dysfunction. Cardiac and renal responses, defined in agreement with consensus, biomarker-based criteria [21], were seen in 57% and 60% of evaluable patients, respectively. While the study was not designed to assess efficacy and did not include a comparative control arm, these results were considered to compare favorably with respect to historical data sets [47].

Based on these positive results, two multicenter, randomized, double-blind, placebo-controlled, two-arm, efficacy and safety studies were started with birtamimab 24 mg/kg every 28 days as the experimental arm. The PRONTO study (NCT02632786) was an international, phase IIb study including AL patients who had at least a partial hematologic response before and after 16 weeks of therapy. The primary endpoint of the study was the rate of cardiac response, while secondary endpoints included, among others, the rate of kidney and liver best response and functional measures. A total of 129 patients were randomized and could be evaluated (66 in the birtamimab arm and 63 in the placebo arm). There was no significant difference in the occurrence of serious adverse events in the two treatment arms. In an intention-to-treat analysis, there was no statistically significant difference in cardiac response, which was reported in 26/66 (39.4%) patients in the birtamimab arm and 30/63 (47.6%) patients in the
placebo arm (p = 0.319) (Table 3). None of the prespecified, secondary endpoint analyses showed any signs of activity of the experimental drug, including renal and liver responses in evaluable patients, the physical component score of the Short-Form 36 (SF-36) questionnaire, and the 6MWT [48].

The VITAL study (NCT02312206) was a global phase III trial including newly diagnosed AL amyloidosis patients with cardiac involvement who were concomitantly receiving bortezomib-based frontline therapy (with a number of cycles of frontline therapy and eventual further lines of treatment at the discretion of the treating physician, as per standard of care) [49]. Patients eligible for and planning to undergo autologous stem cell transplant were excluded. The primary endpoint of this trial was time to composite of all-cause mortality or cardiac hospitalization, while the secondary endpoints included heart, kidney, and liver best response, functional measures, and patient-reported quality-of-life data (Table 2). Treatment was administered every 28 days until study completion, as this was an event-driven study. A total of 260 patients (130 patients per treatment arm) were enrolled. This study (along with other clinical trials on birtamimab that were open at that time) was terminated by the sponsor based on a futility analysis, whose results were disclosed shortly before the results from the PRONTO study. Death or cardiac hospitalization occurred in 56/130 (43.1%) patients in the birtamimab arm and 62/130 (47.7%) patients in the placebo arm, and there was no statistically significant difference in the composite primary endpoint (log-rank test: p = 0.3300; hazard ratio [HR] 0.835, with two-sided 95% confidence interval [CI] 0.5799–1.2011). Again, no specific safety signal emerged during the study, with similar rates of serious adverse events in the two study arms (Table 3). Data on secondary outcome measures have not yet been reported.

Based on a non-prespecified subanalysis on patients presenting with advanced amyloid disease (Mayo stage IV [50]) at baseline, showing a significant survival benefit favoring birtamimab in these patients, with 74% of birtamimab-treated patients alive at 9 months versus 49% of patients in the control group, a confirmatory, multicenter, global, randomized, double-blind, placebo-controlled phase III trial (the AFFIRM-AL study, NCT04973137) was started in patients with Mayo stage IV [51, 52]. In this study, birtamimab (24 mg/kg) or placebo will be administered every 28 days to newly diagnosed, Mayo stage IV AL amyloidosis patients receiving standard-of-care, bortezomib-containing therapy (± the anti-CD38 monoclonal antibody daratumumab at the discretion of the investigator). Patients with NT-proBNP levels exceeding 8500 ng/L or concomitant multiple myeloma are excluded from this trial. A total of 150 patients (100 in the birtamimab arm and 50 in the placebo arm) are planned to be enrolled. The primary endpoint of the study is time to all-cause mortality, while functional measures (the physical component part of the SF-36 questionnaire and the 6MWT) will be evaluated as secondary endpoints. Of note, the sponsor has reached a special protocol assessment (SPA) agreement with the FDA at fixing a p-value ≤ 0.10 (instead of the canonical 0.05) as the prespecified, significance threshold for the statistical analysis of the study outcome [52]. The study was started at the end of August 2021 and the estimated study completion date is June 2024. Results from this study are eagerly awaited.

### 4.2.2 Anselamimab

The monoclonal IgG1 antibody (mAb) anselamimab (CAEL-101) is the chimeric form of murine mAb 11-1F4, which was developed by Solomon’s group back in 2003 [53]. The antibody was produced by injecting, in BALB/c mice, the Vκ4 fragment derived from endopeptidase cleavage of a κ4 Bence Jones protein [54].

Subsequent studies indicated that the mAb 11-1F4 bound to a conformational neoepitope within the first 18 amino acids of misfolded human immunoglobulin LCs [53, 55], while it did not recognize native, circulating LCs. In vivo, the mAb expedited dissolution of AL amyloidomas in mice through the recruitment of neutrophils and activation of macrophages [54].

The specificity of the mAb was confirmed via immunohistochemical and radiographic detection by PET/CT of human AL amyloid deposits in patients who received the mAb intravenously [56].

These results led the National Cancer Institute’s Biological Resource Branch to production of the anti-amyloid fibril chimeric IgG1 antibody anselamimab (CAEL-101). The outcome of the phase Ia/b trial (NCT02245867) has been recently reported [57]. This was an uncontrolled, open-label, single-center, ‘up-and-down’ dose-escalation study investigating the tolerance, safety, pharmacokinetics, and possible clinical benefit of anselamimab (Table 2). Patients with measurable, localized, or systemic AL amyloidosis with relapsed/refractory disease were eligible. Dialysis, Eastern Cooperative Oncology Group (ECOG) performance status >3, and seriously limited cardiac, renal, or hepatic function were among the key exclusion criteria for this study. Single-patient cohorts were planned to receive either single infusions (phase Ia) or four weekly infusions (phase Ib) of the study drug starting at 0.5 mg/m² and progressively increasing up to 500 mg/m². If a patient experienced dose-limiting toxicity, then two additional patients were enrolled at the same dose, and the maximum tolerated dose was then defined as the highest dose at which two patients had no dose-limiting toxicity.

In the phase Ib study, the two highest doses were planned to be expanded to investigate dose response. A total of 27
patients were enrolled, all with systemic AL amyloidosis (8 in phase Ia and 19 in phase Ib). The median time since exposure to the last anti-plasma cell therapy was 2.6 and 7.4 months (range 0–15.5 months) in phases Ia and Ib, respectively. Of note, 5/8 patients in phase Ia and 15/19 patients in phase Ib had achieved at least a very good partial response or better to the anti-plasma cell therapy. No dose-limiting toxicity was registered up to 500 mg/m² and the maximum tolerated dose was not achieved in either phase Ia or Ib. Grade 3 or higher adverse events included pruritus and pericardial effusion, reported in one patient each. One patient developed a mild, transient, and spontaneously resolving rash 4 days after a single dose of anselamimab 50 mg/m², and a skin biopsy revealed a previously undiagnosed cutaneous amyloidosis with amyloid deposits that proved positive for the 11-1F4 antibody. Pharmacokinetic studies indicated a biphasic disposition of the antibody due to a rapid distribution phase, slower elimination phase, and long half-life of 10–16 days, consistent with the pharmacokinetics of other IgG antibodies, but in contrast with the 4–16 h half-life of dezamizumab (Table 1). Excluding three patients without measurable disease, the overall organ response rate was 63%, with a median time to response of 3 weeks [57]. Organ responses were noted only among evaluable patients receiving > 5 mg/m² of anselamimab. A cardiac response was detected in 8/12 evaluable patients (67%) and was accompanied by a statistically significant improvement in global longitudinal strain, while the remaining patients had stable cardiac disease. A renal response was observed in 2/10 evaluable patients (20%), while 60% had stable disease and 20% experienced renal progression (Table 3). The organ response should be interpreted with caution because 7/8 patients with cardiac response and 1/2 patients with renal response had obtained a deep hematological response from previous chemotherapy, and a concurrent benefit on organ function due to profound reduction of the proteotoxic LC cannot be ruled out.

Anselamimab is under further clinical development, with a phase II (NCT04304144) trial and, notably, two phase III trials (NCT04512235 and NCT04504825) in patients with advanced amyloid cardiac involvement. The outcome of these two trials will be extremely informative on the possibility to recover organ function in patients who present with advanced organ damage.

4.3 Targeting the Amyloid Deposits in ATTR Amyloidosis

4.3.1 PRX004

Potential therapeutic amyloid-directed antibodies have been developed by several groups using a rational, structure-based approach, targeting epitopes that are inaccessible in TTR tetramers and exposed in the monomers or non-native conformations of TTR. This feature allows the targeting of amyloidogenic TTR species while preserving the functions of the native protein [58–60]. Using this rationale, structure-based, approach to generate mAbs that selectively targeted a TTR cryptotope, composed of residues 89–97, four mAbs were produced, showing high affinity and specificity for misfolded TTR species [60]. Mice were immunized with the TTR peptide 89–97 and screened for specific binding to non-native TTR conformations, suppression of in vitro TTR fibrillogensis, promotion of antibody-dependent phagocytic uptake of misfolded TTR, and specific immunolabeling of ATTR amyloidosis patient-derived tissue. Four antibodies were selected, and the possibility to exploit these antibodies for the diagnostic imaging of amyloid deposits was also envisaged [60].

One of the four murine moAbs (which one was never disclosed by the Pharma company) was humanized and tested as PRX004 in an uncontrolled, international, multicenter, phase I, dose-escalation study (NCT03336580) assessing safety, tolerability, pharmacokinetics/dynamics, and immunogenicity of PRX004 (Table 2) [61]. The target population was represented by patients with ATTRv amyloidosis. Concomitant treatment with the TTR tetramer stabilizers tafamidis or diflunisal was permitted, provided that the dosing was stable in the previous 6 months, while prior or planned liver transplantation or recent exposure to TTR-silencing agents patisiran or inotersen was not allowed. This trial included a dose-escalation phase with a 3 + 3 design, exploring the range of up to three infusions of PRX004 at doses of between 0.1 and 30 mg/kg (at 28-day intervals), followed by an expansion phase and a long-term extension phase for up to 15 infusions. A total of 21 ATTRv patients completed the dose-escalation phase and 17 patients were enrolled in the long-term extension phase. No treatment-related serious adverse events occurred. Adverse events occurring in more than 10% of treated patients included fall, anemia, upper respiratory tract infection, back pain, constipation, diarrhea, and insomnia. The pharmacokinetics profile of PRX004 was consistent with IgG1 monoclonal antibodies. Although the study was not designed to assess efficacy, preliminary efficacy data were reported for seven evaluable patients (pooling data from 3, 10, and 30 mg/kg doses groups under the assumption that dose levels ≥ 3 mg/kg should in principle enable clearance of > 90% of amyloid deposits). An improved global longitudinal strain (mean change: − 1.21%) was reported in all seven evaluable patients and an improved NIS was seen in 3/7 cases (Table 3). All seven evaluable patients were reported to have a slowed disease progression compared with natural history data.

A phase III study is under planning by the new pharma company that continued the development of the drug [62].
4.3.2 NI006

The human monoclonal antibody Ni301A was developed using high-throughput screening of human memory B-cell libraries, which generated a library of ATTR-binding antibodies, selected for high-affinity binding to ATTR, amyloid removal activity, and absent binding to native TTR [59]. This functional selection process led to the identification of Ni301A. Ni301A binds to the linear epitope WEPFA, located in position 41–45 of mature TTR protein, hidden in TTR’s naturally folded conformation but accessible to antibody binding following unfolding and aggregation. Ni301A binds ATTR deposits in human cardiac tissues and triggers ATTR removal from the post-mortem tissues ex vivo via phagocytosis through added human macrophages. In wild-type mice grafted with patient-derived ATTR fibrils, systemically administered Ni301A accumulates on the grafts and rapidly removes them via Fcγ-mediated phagocytosis.

Ni301A, with the drug name NI006, is now being tested in a phase I clinical trial (NCT04360434).

5 Future Directions

The therapy of systemic amyloidoses has achieved outstanding results in the last few years, with the unprecedented approval worldwide of three drugs and one treatment regimen, which have changed the natural history of these diseases. The treatment of these protein misfolding diseases is deeply enrooted in the molecular cascade of events that underlie their onset and progression. The reduction of the availability of the misfolded amyloid protein precursor, either by suppressing the synthesis with chemotherapy/immunotherapy in AL amyloidosis or by gene silencing/editing, or stabilization of the kinetically unstable transthyretin homotetramer, is the most effective strategy that prolongs progression-free survival, quality of life, and overall survival [14]. However, the recovery of the target organ function, particularly the heart, occurs in fewer than half of the patients and is a lengthy process requiring a median time of 12 months to achieve best cardiac response in AL amyloidosis [63]. Patients without very advanced cardiac damage have time to benefit from the slow recovery process that follows the eradication of the amyloid precursor, while patients who present with severe cardiac involvement are short of time. There is hope that the accelerated clearance of the amyloid fibrils would halt their cytotoxic effects and restore the tissue architecture and function, opening a therapeutic window also in patients presenting with severe heart damage. The outcomes of the two randomized trials in AL amyloidosis comparing bortezomib-based therapy versus bortezomib-based therapy plus anselamimab in patients with stage IIIa and IIIb cardiac involvement (NCT04512235 and NCT04504825) are eagerly awaited. These trials will shed light on the critical question regarding the threshold of cardiac damage that determines the irreversibility of heart failure and will inform the development of novel anti-amyloid agents.

An important area of research is represented by the identification of novel operational targets on amyloid fibrils and new targeting constructs with adequate specificity, to reduce off-target adverse events, and biodistribution, to ensure full access to the deposits. With the recent advances in solid-state nuclear magnetic resonance (NMR) and cryo-electron microscopy (cryo-EM), obtaining structural details of ex vivo amyloid fibrils is now possible [64–66]. The definition of the remarkable structure of B-cell marker CD20 in complex with rituximab [67], illustrates the potential of cryo-EM to bring rational drug design approaches to therapeutic antibodies [68].

Therapeutic strategies combining drugs with different and synergistic mechanisms of action, such as those reducing the availability of the amyloid protein precursor with those that accelerate the clearance of the deposits, will probably enter the clinical scenario in the near future. Further research is necessary to define the best treatment scheme (parallel or sequential combination) and for the development and validation of much-needed accurate biomarkers of progression and treatment response. The availability of validated biomarkers as qualified surrogate endpoints [69, 70] would accelerate the development of new drugs through innovative trial designs that include relevant patient-centric endpoints, such as patient-reported outcomes.

6 Conclusion

Anti-amyloid therapies hold great potential to significantly impact the natural history of systemic amyloidoses in conjunction with anti-precursor therapies. There is great expectation that the several ongoing trials will provide a solid answer to the key question “Do amyloid-lowering drugs help patients?”, which is still open in Alzheimer’s disease [71]. The progressive elucidation of the molecular mechanisms of systemic amyloidoses opens new therapeutic opportunities and several new drugs are in the pipeline. Considering the high cost of the drugs used in rare diseases, it will be imperative to assure accessibility to all patients [72, 73] through dialog between investigators, pharmaceutical companies, patient advocacy groups, and regulatory agencies.

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**Author contributions** GM conceived the study and drafted the initial version of the manuscript. All authors contributed to the literature research, data analysis, and writing. All authors read and approved the final manuscript.

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