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Review

Learning from Patients: The Interplay between Clinical and Laboratory Research in AL Amyloidosis

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Abstract: Primary systemic light chain amyloidosis (AL) is a rare monoclonal plasma cell disorder. Much research has been performed to determine the factors that underly amyloidogenicity. However, there is increasing evidence that the primary clone, and also patient-related factors, influence the mechanism and rate of the process. The lessons learnt from patient care definitely imply that this is not solely due to the deposition of material in the tissues that cause organ injury but amyloid light chain precursors are likely to mediate cellular toxicity. The disease rarity, combined with the lack of in vitro tools, and that multi-organ failure has a wide clinical spectrum, result in investigative challenges and treatment limitations (due to AL patient frailty). All these characteristics make the disease difficult to diagnose and indicate the need to further study its origins and treatments. This review will focus on the various aspects of the amyloidogenic plasma cell clone, as learnt from the patient care and clinics, and its implications on basic as well as clinical trials of AL research. Details regarding the etiology of the plasma cell clone, understanding the diagnosis of AL, and improvement of patient care with specific consideration of the future perspectives of individualized patient therapy will be described.

Keywords: AL amyloidosis; light chain toxicity; plasma cells

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1. Introduction

To paraphrase the first sentence of Lev Tolstoy's novel Anna Karenina: "Happy families are all alike; every unhappy family is unhappy in its own way", we may say: "Normal plasma cells are all alike; every amyloidotic plasma cell is dysfunctional in its own way." In this respect, it is remarkable to note that each patient has a different disease—different pace, organ involvement, extent, symptoms, and even response to the treatments. This makes amyloidosis an even rarer disease that is so difficult to study.

There are multiple levels of challenges: how pathological is the clone in contrast to the pathologic protein? Is primary amyloidosis (AL) just a misfolded protein monoclonal gammopathy of unknown significance (MGUS) type of disease, or does the plasma cell clone have a unique etiology and therapeutic importance? Can we recognize the true pathological clone—at the plasma cell level or the protein level? Are patients with multiple myeloma (MM) the same as AL patients? Lessons learnt and accumulating from the clinics and laboratory show the amyloid producing plasma cell clones are unique. When we have an MGUS patient with cardiac or other symptoms, how can we diagnose AL? How can we utilize the cooperation with patients to achieve better results and subsequently individualize patient therapy? How can we bring all of these challenging questions to the laboratory and hope for practical answers to these issues, not just hypothetical ones, and transfer these answers from the bench to better practice in the clinic?

2. The Amyloidogenic Clone

All plasma cell dyscrasias are characterized by the proliferation of monoclonal plasma cells that produce a monoclonal protein [1,2]. Whereas MGUS and MM are common,

primary AL is rare among the three plasma cell dyscrasias. MGUS is a premalignant disorder with no end organ damage [1,3], and approximately 1% of patients progress to MM each year. When the plasma cell bone marrow load is higher, i.e., above 10%, the MGUS becomes smoldering MM (SMM) and the chance of progression to overt active MM is much higher at 10 to 20% per year. Unlike MGUS or SMM, active MM is a malignant disorder associated with a high plasma cell burden in the bone marrow [2,4]. In contrast to MM or SMM, patients with AL have a small plasma cell clone that secretes misfolded and unstable monoclonal light chains that can aggregate and form systemic extracellular deposits of amyloid fibrils, causing toxic organ damage [5–7]. When vital organs are targeted by the amyloidogenic light chains, it ultimately leads to the patient's death without specific therapy. Cardiac involvement is very common (60–70% of patients) and confers the gravest prognosis, with 3 to 6 month overall survival without treatment [8]. Thus, patients die because of the toxicity of amyloidogenic light chains and amyloid fibrils and not because of the plasma cell clone proliferating [7]. Each patient has a unique disease-causing light chain, explaining at least in part the conspicuous heterogeneity of the clinical picture and making AL a truly protean disorder.

The precursor B cell was not thought to be different between MGUS, MM, or AL. However, reports have recently [9,10] demonstrated a higher frequency of specific chromosomal aberrations in the plasma cells of AL patients compared to MGUS or MM patients; an example is the [11,12]. The presence of different cytogenetic aberrations may imply that AL pathogenesis is not solely attributed to the LC properties. This is also true phenotypically. The characterization of the AL plasma cell clones by flow cytometry was able to distinguish these cells from the PCs of MGUS and MM [11]. Multidimensional-flow-cytometry detected clonality in almost all AL patients with extremely high sensitivity. Furthermore, an automated risk-stratification system based on plasma cell flow cytometry features was able not only to identify those patients but also to independently recognize a prognostic impact on progression-free and overall survival of AL patients [13]. In contrast, a recent whole exome sequencing of AL patients' plasma cells supported the notion that the germline immunoglobulin gene is a key determinant in the pathogenesis of AL, predisposing the light chain from the plasma cell clone to adopt an aberrant conformation typically closer to the plasma cells of patients in the MGUS stage before the development of a higher tumor burden and the accumulation of MM driver mutations [13]. Single cell genomic analysis allowed a sensitive characterization of 40 individual patients', including four AL patients', entire genetic spectrum of their abnormal plasma cell clone [14]. A similar but newer study [12] showed that the plasma cells of AL share greater similarity to earlier "lymphoid organs" plasma cells, whereas the plasma cells of MM are transcriptionally closer to more mature "peripheral blood" plasma cells, the former again relating to a more MGUS-like genotype. Other genetic features that were found showed that a certain genomic transcriptional signature correlated with upregulation in protein *N*-linked glycosylations and others with inferior survival in AL. In this respect, there is growing genetic evidence that, although the amyloidgenic plasma cell clone resembles more closely than the MM proliferative plasma cell clone the MGUS plasma cell clone, it still has its own distinct features unique to the phenotype of the amyloidogenic clone itself and features that may predict the prognosis.

3. The Clonal Distinction of AL PCs May Aid in Diagnosis

The early discrimination between benign and malignant plasma cell dyscrasia is crucial to prevent irreversible injury, yet, paradoxically, end-organ damage is required for the recognition of the presence of malignant plasma cell dyscrasia [15]. Patients with active MM are usually diagnosed easily because their end-organ damage is typically symptomatic (anemia, bone pain, hypercalcemia, and renal failure). In contrast, in AL, the only abnormality, the presence of monoclonal free light chain, serves also as the diagnostic marker of benign plasma cell dyscrasia, thereby delaying early diagnosis, leading to significant end-organ damage prior to the diagnosis (heart and kidney failure, etc.) [16].

Moreover, this end organ damage induced by AL, unlike in MM, is common to many other diseases and highly nonspecific, necessitating extensive workup for full diagnosis [6,17]. A survey completed by 533 participants revealed that the diagnosis of AL was not established until $\geq$1 year after the onset of initial symptoms and received by a hematologist only after $\geq$5 visits to various other physicians, thereby exemplifying that establishing the diagnosis of AL is difficult [18]. These data support the need for better early identification and support for patients seeking a correct diagnosis [18,19]. Increasing clinician awareness may reduce the time to diagnosis, and additional research is needed to identify optimal diagnostic testing to reduce delays in treatment initiation and the subsequent severe impacts on the patients' health.

Clinical features are, therefore, the mainstay of the initial diagnostic process. These clinical features, as outlined in Table 1, are the signs and symptoms that should raise the suspicion of AL to the general practitioner and specializing physicians in different fields of expertise, including hematologists. This early identification of the disease can serve as a major component not only regarding the survival of patients but also in terms of their quality of life [20]. The burden of AL diagnosis, especially in the cardiac patient subgroup, tends to be greater on physical health than on mental health. Furthermore, as MGUS is extremely prevalent in the general population, so are other diseases (hypertension and diabetes mellitus) that lead to organ damage, simulating the symptoms of AL, such as renal failure, proteinuria, and left ventricular hypertrophy. In addition, it is rare that high levels of monoclonal protein can be found by standard methods, so alternative methods need to be sought [21]. The classical serum and plasma biomarkers, such as conventional cardiac markers (N-terminal pro B-type natriuretic peptide and troponins), are also not specifically diagnostic. There are other surrogate markers associated with AL reported, such as growth differentiation factor-15 levels, which improve the prognostication in patients with cardiovascular and renal disorders and are associated with a high risk of progression to dialysis [22]. Again, it is not specific for AL patients. Others, such as von Willebrand factor and ADAMTS-13 antigens, can be elevated as compared with healthy controls [23], as well as some serum angiogenic cytokines [24], but these are not specific for AL patients either. The prediction of AL in the workup of MGUS patients with a clinical suspicion of AL, based on the (Table 1) clinical features, may, therefore, be challenging.

Table 1. Clinical features that must raise a suspicion of AL to the general and specializing physician.

AL amyloidosis is a multi-organ involvement disease. The presence of many unexplained different symptoms in a single patient is alarming
Cardiac hypertrophy without the expected corresponding high voltage by ECG
Proteinuria and albuminuria with no chronic kidney disease and large- or normal-sized kidneys by ultrasonography
Proteinuria and albuminuria in the background history of hypertension or diabetes mellitus but the absence of the expected disease's retinopathy
Peripheral edema with no causal disease
Skin ecchymoses and other bleeding tendency with no coagulation or other causal disease
Peripheral polyneuropathy with no causal disease
Gastrointestinal complaints, especially accompanied by weight loss with no causal disease
Bilateral Carpal Tunnel Syndrome
Any of these features in an MGUS or SMM patient

AL patients have immune dysregulation as manifested by the intact immunoglobulin immunoparesis. This immunoparesis was reported as an independent prognostic factor for AL and response to treatment [25]. In this respect, we hypothesized that immunoglobulin heavy/light chain (Hevylite®) isotype patterns may help discriminate between AL and benign plasma cell disorder states [26]. The heavy light chain assays separately quantify

the different heavy chain forms of each immunoglobulin isotype. By multivariate analysis, the heavy light chain was found to be the best independent test predictor of AL [26]. Heavy light chain suppression had an odds ratio of 15, and elevation had an odds ratio of 10; thus, they were significant variables in the diagnosis and exclusion of AL [26]. Furthermore, patients with both heavy light chain suppression and no elevation had an odds ratio of 317 to be diagnosed with AL rather than a benign plasma cell disorder [26].

The unique features of the clone and the proteosomal amyloidogenic changes observed in AL patients' plasma cells and serum free light chains (i.e., the light chains not accompanied by the corresponding immunoglobulin heavy chains that circulate in the serum) are not only a variation allowing the understanding of the pathogenesis of the disease but also a hallmark of differentiating the disease from other plasma cell disorders, and can even aid in the differential diagnosis of a new patient under investigation for AL. The unique feature of AL light chains is their misfolded secondary and tertiary structures [6,27,28]. There is an inherent proteomic variance of the amyloidogenic free light chain, which is not only abnormally elevated but also tends to have high dimerization levels. Analyses of these dimerization patterns show different properties between malignant and benign types of plasma cell disorders [28]. This was shown in a small subset of MM and AL patients compared with MGUS patients [28]. We further conducted a direct study to validate these dimerization patterns, which allows a distinction between AL and benign plasma cell disorders patients [29]. The sensitivity of the analysis was found to be 92.5%, with a remarkable negative predictive value of 91.9% [29]. The test, which is western blotting-based, can be operator-dependent and tedious. Nevertheless, it may differentiate between benign plasma cell disorders and AL and prevent the usage of more laborious as well as invasive procedures.

With the advancement in the ability to characterize the aberrant light chain by mass spec proteomics [30–32], it has been shown that matrix assisted laser desorption ionization time-of-flight mass spectrometry, termed MASS-FIX, which utilizes the detection of the isotype of the serum monoclonal protein, has the ability to identify the light chain N-glycosylation. This monoclonal light chain glycosylation is a potent risk factor for progression to AL and may, as such, also be used to predict both the presence and progression to light chain AL.

As shown by flow cytometry [11], a distinct immunophenotype feature of the aberrant plasma cell may be utilized in the differential diagnosis of AL. This is also evident by the assessment of the genomic transcriptional features of the plasma cell genotype [12]. Although these show high sensitivity and specificity, they necessitate an invasive BM examination and the ability of experienced and specializing laboratories, as well as expensive and sophisticated assays. These may become the future of highly accurate screening diagnostics but are not currently and readily available.

In these respects, there is growing proteomic evidence coming from assays measuring various markers and light chain changes and patterns, allowing not only the discrimination and characterization of the amyloidogenic light chain and plasma cell clones but also to differentiate the MGUS patients from the AL newly diagnosed patients and, furthermore, predict the prognosis.

4. The Plasma Cell Clonal Features Aid in the Individual Patient Treatment

Bortezomib/dexamethasone-based therapy (usually with the addition of a third agent, such as cyclophosphamide or melphalan) is the standard first-line therapy for AL, but only 30–40% of patients can achieve a rapid deep response (i.e., $\geq$VGPR) within the first three cycles [16,33–35]. Extensive work has been performed to understand the importance of proteasome inhibition effects on amyloidogenic light chain clones [36]. Patient primary cells with AL, as compared with MM plasma cells, have higher apoptosis rates and a lower proliferative index [37]. The AL plasma cells are associated with distinctive organelle features, such as expanded endoplasmic reticulum perinuclear mitochondria and higher numbers of stress-related transcripts. [38]. AL light chain expression alters proteostasis and

cell growth [38]. This apoptotic phenomenon, combined with autophagy, was also shown to be a major mechanism of bortezomib sensitivity in AL cells [38]. In comparison with primary MM plasma cells, AL plasma cells engineered cell lines revealed an unprecedented intrinsic sensitivity to bortezomib, even higher than that of MM plasma cells, associated with distinctive organellar features and expression patterns indicative of cellular stress. These consisted of an expanded endoplasmic reticulum, perinuclear mitochondria, and a higher abundance of stress-related transcripts and were consistent with reduced autophagic control of organelle homeostasis [38].

In second-line treatment, Daratumumab-based therapy with or without lenalidomide and dexamethasone are the standard of care regimens. Yet, only 60% achieve VGPR or better and 10 to 40% CR [39]. As expected, AL plasma cells express on their surface CD38, a type 2 transmembrane molecule that acts as both a receptor and an ectoenzyme. Levels of CD38 expression affect the CD38 antibodies mediated immune response and cell killing in vitro. Plasma cells s of patients with AL do not homogenously express high CD38, yet higher CD38 expression in AL is associated with the worst event-free survival, probably because it is associated with more severe cardiac involvement, as shown by higher serum N-terminal pro B-type natriuretic peptide concentrations [40–42]. These features are in line with both the pre-clinical and the impressive clinical results rendering AL patients' plasma cells and AL patients' disease sensitive to daratumumab [35,43,44]. Recently, the Andromeda phase three first-line trial has shown that the significance of achieving a CR, and especially a deep response with a dfee light chain < 10 mg/L, translates to a sustained and extremely favorable hematologic and high organ response [45]. However, even when daratumumab and cyclophosphamide are used as a first-line therapy, only 53% of the patients achieved CR and 78% VGPR. Thus, for a significant number of patients, this combination therapy is insufficient.

A critical characteristic of the plasma cell clone in AL is that, while typical MM high risk cytogenetics features, such as del17p and t(4;14), are rare in AL, between 40 and 50% of the patients harbor plasma cell clones with the cytogenetic feature t(11;14) [9,10,46], while only 15% of MM patients will have this translocation. AL patients are also likely to express BCL2. While the presence of t[11;14] is considered a neutral (i.e., non-high-risk) prognostic factor for survival in MM patients [46], in patients with AL, there are data indicating that the presence of t[11;14] is associated with poorer outcomes and has been associated with less optimal responses to bortezomib and dexamethasone [17,34,47]. Hence, a therapy that is specifically targeted for patients with t[11;14] is highly needed for patients with AL.

Venetoclax is a potent, selective, orally bioavailable small-molecular inhibitor of BCL-2 [48]. Venetoclax selectively binds to BCL-2, displacing pro-apoptotic proteins and leading to mitochondrial outer membrane permeability, causing the activation of caspases and subsequent restoration of apoptosis. In MM, early studies have shown efficacy in heavily pre-treated patients [49–51]. Nevertheless, the most significant efficacy has been observed in patients with the genetic translocation t[11;12], and, combined with dexamethasone in relapsed/refractory MM, patients have demonstrated a synergistic effect [52]. Given the fact that almost 40 to 50% of patients with AL harbor a t[11;14] translocation, it is hypothesized that this compound will be a future major player in AL patient treatment. There are a few small reports published on the safety and efficacy of venetoclax in AL patients [53–55]. Despite the wide variety in venetoclax treatment (i.e., dosing), of the eight evaluable AL patients, three achieved CR and four achieved a VGPR, resulting in an 87% overall response rate [55]. A recent larger retrospective series [56] of 43 patients with AL harboring t[11;14] has shown a remarkable activity, with high hematologic responses (81%) and very high VGPR/CR rates (78%), and progression free survival and OS that were not reached at the time of last follow up. These initial results are encouraging, setting the stage for larger scale, prospective clinical trials.

To summarize, the AL amyloidogenic clone has unique features different from its MM plasma cell counterpart clone. Because of the intrinsic effects of the amyloidogenic plasma cells, excess endoplasmic reticulum stress, and autophagy, AL patients respond

better to proteasome inhibition. Furthermore, CD38 expression makes the AL plasma cells exquisitely sensitive to daratumumab and the presence of t[11;14] (which confers worse prognosis); this is becoming an advantage because of the advent of the targeted therapy venetoclax.

5. The Amyloid Deposition and Novel Strategies for Removal and Their Significance

While novel anti-plasma cell therapies, such as proteasome inhibitors, immunomodulators, monoclonal antibodies targeting a surface protein on the plasma cells, and the molecular inhibitor venetoclax, are the mainstay of therapy, there is still a dire need for therapies that directly target the amyloid fibrils and reverse organ damage. NEOD001 is a humanized IgG1k monoclonal antibody directed against an epitope on amyloid fibrils that binds with a high affinity in a conformation dependent manner to misfolded light chains. Although there were some favorable initial results, the final phase two (PRONTO) and 3 (VITAL) clinical trials have failed to show a significant clinical outcome [1]. However, a post hoc analysis focusing on advanced cardiac AL patients, looking at overall survival without regard to cardiac hospitalizations, showed a potential benefit, with a hazard ratio of 0.498 (95% CI 0.24–1.03) $p = 0.055$, among 77 Mayo stage IV patients [2]. CAEL-101, formerly known as 11-1F4, is an IgG1k monoclonal antibody that binds directly to the conformational epitope present on human light chain amyloid fibrils regardless of the κ or λ isotype. In a recently published phase one trial, 15 of the 24 patients (63%) who manifested cardiac, renal, hepatic, gastrointestinal, or soft tissue involvement had a therapeutic response to monoclonal antibody CAEL-101, as evidenced by serum biomarkers or objective imaging modalities with median time to response of 3 weeks. Infusions of monoclonal antibody CAEL-101 were well-tolerated and, for the majority, resulted in improved organ function, notably for those with cardiac impairment [3]. Given these results, upcoming anti-amyloid fibril antibody studies exploring the opportunities to improve outcomes for the sickest AL patients with advanced cardiac disease may improve survival and reduce the risk of early death in this uniquely frail population [2].

6. The Patient's Burden of AL on Health-Related Quality of Life

The effects of treatment are extremely important not only for the outcomes of organ responses and overall survival of AL patients but especially regarding their quality of life [57–59]. This is another important aspect to be taken into consideration in patient care that may be quantified and followed. Lessons learnt from the quantification of quality of life helps physicians identify ancillary treatments and services geared towards improving patients' functioning, well-being, and overall health-related quality of life [20]. These findings also help to support the use of health-related quality of life end points as important outcome measures in current and future treatment studies. Asking AL patients to rate their fatigue and quality of life had predictive value, indicating that assessing pre-treatment levels of quality of life, particularly related to physical well-being, may be a significant prognostic factor regardless of the treatment received [58]. Not only was survival at one year associated with significant improvement in quality of life but baseline patient reported fatigue was also an independent prognostic factor for overall survival [59].

7. From the Patient to the Bench Side: The Case for LC Toxicity and Other Laboratory Data Influenced by Data Accumulating from Patient Care

Unlike in MM, the achievement of a fast and deep hematologic response in AL is the sole factor to correlate with better survival and organ responses [60,61]. Lessons learned from the clinic reveal that reducing the concentration of the circulating amyloidogenic free light chains improves cardiac function and prolongs survival [62,63]. The median overall survival is longer in patients with dfree light chains < 10 mg/L, and those achieving CR. Multivariate analysis showed the presence of CR as the most important independent prognostic factor for survival. This was promptly proven by the encouraging results of the Andromeda trial [45], where deep responses were directly correlated with organ

responses, e.g., the deeper the response, the better the ability of the organs not only to not deteriorate and stabilize the affected organs but also to rehabilitate them. However, the remaining toxicity in treated patients can be due to the long persistence of amyloid deposits in organs because of their high resistance to proteolysis. Moreover, the detection of minimal residual disease by flow cytometry after treatment was found in approximately half the AL patients in CR and correlated with treatment organ responses. AL patients with undetectable minimal residual disease have a very high probability of organ response and a very low probability of hematologic relapse. Persistent minimal residual disease may explain persistent organ dysfunction [64,65].

How can we explain these findings in light of the fact that these minimal amounts of marrow plasma cells and circulating and light chain still cause damage, and that these dysfunctions may happen without a change in organ size or the amount of amyloid deposits in the damaged organ? These indicate that it is not solely the deposition of the amyloid mass in the tissues that causes organ injury [66–68] and that intrinsic light chains toxicity is a fundamental contributor [7]. Unlike in MM, where the clinical picture is dominated by the hyper-proliferative MM clone, in AL, severe organ dysfunction is usually caused by a small plasma cell clone producing the amyloidogenic light chain [7]. In AL, the plasma cell clones are usually small (median 10% of bone marrow cells) and, interestingly, the free light chains levels in AL may be significantly lower than in MM, irrespective of serious organ damage and the binding site [69–74]. It is evident that AL patients with higher plasma cell burdens have worse prognoses [69]. Previous studies have shown that AL patients' plasma cells have low proliferation and high apoptotic rates as compared with monoclonal gammopathies patients' plasma cells and MM patients' plasma cells [37]. Furthermore, a human-derived cardiomyocyte cell line, when exposed to recombinant amyloid fibrils, internalized them, resulting in growth arrest—specifically, mitochondrial dysfunction [75]—and exposure of AL fibrils to cardiomyocytes induces changes in the pathways associated with immune response and extracellular matrix components [76]. Additionally, the urinary extracellular vesicles in patients suffering from AL of the kidney contained monoclonal lambda light chains [75]. Benedikt et al. found that specific mutations in the variable domain regions ability to fold can have an effect on light chain domain interactions, promoting stability and amyloidogenicity [77]. The revised [70] MAYO classification has added free light chains levels to be a part of the initial prognostic assessment; nevertheless, it is also interesting to note that each patient is different from another in the effects of the light chain toxicity. Excess plasma cells, and, subsequently, the free light chains serum concentration, correlate with the prognosis, yet this is not a linear correlation and is of no correlation to organ involvement. Comparing cardiac to renal involvement, patients reported significant differences in dfree light chains (difference in involved and uninvolved light chain). Organ involvement was a significant predictor of dfree light chains, and renal involvement was associated with lower circulating light chain burden, which cannot be fully explained by marrow plasma cell burden alone [78]. As such, the etiology for these changes may be an increased sensitivity of the kidney to light chains or their excess toxicity. In accordance with the clinical observation that the serum free light chains patterns in AL patients are log lower than those in MM patients, we examined the correlation between the free light chains levels and malignant plasma cell clone size. We found that, irrespective of free light chains levels, the clone size did not correlate with the amount of serum free light chains (Figure 1). Although AL patients' plasma cell content within the bone marrow (assessed by immunohistochemistry) does correlate to the free light chain levels at diagnosis, with a trend found as free light chain levels rose (Figure 1A), this trend was not as evident per sample. When each sample was plotted individually, only a borderline significant significance with low correlation was observed (Figure 1B). Thus, nonetheless, the Ig heavy chain levels, when elevated, do correlate (Pearson R 0.38 with $p = 0.007$) with the bone marrow plasma cells load (not shown). Note also that, contrary to MM, the absolute level of free light chains is less than 1000 mg/L, even in those patients with a very high BM plasma cell count of >50% BM plasma cells. This may also suggest that AL related damage does not necessarily correlate

with the amount of malignant plasma cells or free light chains levels, implying that there is an inherent toxicity irrespective of the concentration of the amyloidogenic light chains themselves. In vitro, a study of the light chains from cardiac and renal patients highlights the concurrency of different biophysical traits linked with light chains' amyloid propensity, suggesting that thermal dynamics correlate with the proteotoxicity seen of each light chain tendency [79].

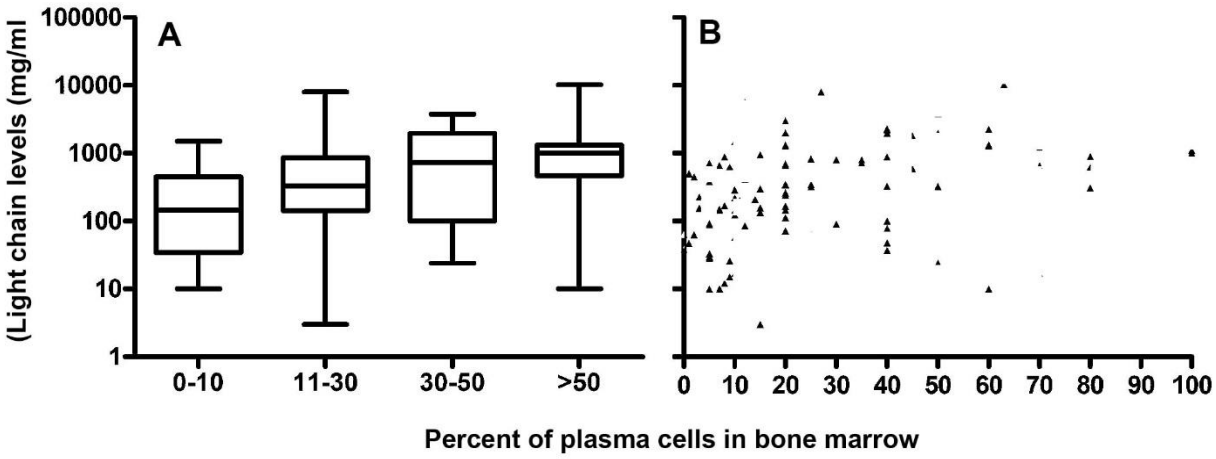

Figure 1. AL patients' plasma cell content within the bone marrow in correlation to the serum free light chain levels at diagnosis. (**A**). Bar plot correlating plasma cells percentage clustered (10%< and >50% $p = 0.001$). (**B**). Dot plot correlating plasma cells percentage with free light chain levels (Pearson r = 0.225. $p = 0.07$).

Genetically, data regarding the light chain rearrangement of the IGVL gene was significantly correlated with the organ tropism [80]. This was further validated to identify the dominant clone, also revealing differing patterns of overall antibody repertoire disruption in different patients [81]. Another novel method to study light chain damage is a machine learning approach [82]. LICTOR is a web-based data analysis predicting light chain toxicity in AL based on the distribution of somatic mutations acquired during clonal selection. LICTOR represents the first method for the accurate prediction of light chain toxicity from their sequence, allowing the timely identification of high-risk patients, such as MGUS subjects, likely to progress to AL.

Being a rare disease, large controlled patient trials are difficult to conduct in primary AL [16,17], and, given the fact that multi-organ failure has a wide clinical spectrum, this also makes these subjects particularly susceptible to treatment toxicity. In the recent era, novel agents used in MM are the mainstay therapeutic armamentarium, but some are very toxic to these AL patients due to their frailty as compared with MM patients. The current treatments are difficult to tolerate and do not substantially improve the quality of life for most patients [18]. Subsequently, understanding the mechanism underlying the molecular differences between these two diseases, which occurs in the same cell of origin, the PC, is crucial towards finding the best therapy that will be more effective yet less toxic to the AL patients. Furthermore, data on heart and kidney tissue infiltrated or damaged by amyloid deposits is lacking, and the mechanisms involved in the organs dysfunction and cellular death caused by the amyloid proteins are poorly understood.

In vitro, many studies are directed towards unraveling the proteomics of the light chain, and analysis of the light chain genetic repertoire has been intensively investigated and related to AL pathogenesis, and to elucidate the mechanism of amyloid fibril formation and even to characterize the amyloid formation in vitro [6,7,27,66,67,80,83–85]. In addition, data on gene expression profiling and proteomics of cells infiltrated by amyloid deposits are scarce [6,38,86,87]. However, proteomic analysis on AL plasma cells themselves identified a higher abundance of keratins in AL plasma cells than normal plasma cells, and, furthermore,

that kappa AL had higher levels of clustering, a protective chaperone, and lower levels of light chains than lambda despite higher levels of circulating light chains [88]. In addition, the characterization of transcriptome changes in a cardiomyocyte cell line RFP-AC16 when cultured with AL fibrils showed an involvement of complement pathway activation and an up-regulation of cytokine and chemokine transcripts [76]. There is a strong debate regarding the mechanism by which AL LC causes organ damage and dysfunction. Amyloidogenic LC precursors are likely to mediate cellular toxicity through a mechanism that causes oxidative stress and activates the apoptotic pathway, thus being directly cytotoxic [27,66].

Various assays have been used to study specific toxicities. Direct cardiotoxicity was characterized by assessing the molecular changes in various cells. Selective organ damage was shown, where dermal fibroblasts [89] were previously shown not to be affected by exogenous toxicity, but damage was evident in primary human cardiac fibroblasts exposed in vitro to soluble amyloidogenic cardiotoxic light chains from AL cardiomyopathy patients [90]. Exposure of the cells to cardiotropic light chains translates into proteome remodeling, associated with apoptosis activation and oxidative stress. The proteome alterations affect proteins involved in cytoskeletal organization, protein synthesis and quality control, mitochondrial activity and metabolism, signal transduction, and molecular trafficking. Other studies showing pro-apoptotic cascades and high oxidative stress caused by the amyloidogenic light chain were conducted utilizing other xenograft models, such as c.elegans and zebrafish [38,91,92].

The role of the microenvironment has also been explored. There is increasing evidence that the biological environment in which aggregation occurs influences the mechanism and rate of the process [6,93]. A summary of the data from genome-wide association studies [94] in search of factors influencing AL risk include genome-wide association studies data from 1129 AL cases and 7589 controls, which predicted that increased monocyte counts, i.e., a causal relationship with monocyte concentration, could be explained by the selection of a light chain–producing clone during the progression of monoclonal gammopathy of unknown significance toward AL. In addition, a potential link to AL amyloidosis pathophysiology of the tumor necrosis factor receptor superfamily member 17 gene was found. Tumor necrosis factor receptor superfamily proteins have key functions in lymphocyte biology.

A long existing challenge has been the lack of a convenient and renewable source of human amyloidogenic light chains [66,68,86]. The purification of primary patient material is limited due to the rarity of the disease as well as technical issues, and generation of recombinant light chains using bacterial expression systems is restricted by the lack of the proper biologic environment, which is instead represented by plasma cells secreting the amyloidogenic light chain. To date, there exists only one characterized amyloidogenic cell line. Its limits are the lack of a completely similar control non-amyloidogenic line and its ability to produce only one light chain type. However, their use is important in assessing the sensitivity to various agents and manipulations to tackle the amyloidogenic plasma cell [95,96]. Cell lines containing AL light chain had higher apoptosis and autophagy. As discussed previously, this apoptotic phenomenon was also shown to be a major mechanism of bortezomib sensitivity in AL cells [38]. We have designed a novel model of multiple myeloma cells expressing AL light chain vitro and found that the amyloidogenic light chain simulated toxicity in PCs and that this toxicity led to increased apoptosis and decreased proliferation due to the malfunction of key biological processes (Pick et al., manuscript under revision). Using RNA-sequencing, the AL light chain producing lines showed higher mitochondrial oxidative stress and decreased activity of the MYC and cholesterol pathways. These studies will allow the assaying of various agents, such as proteasome inhibitors [38], to assess not only the intrinsic effects of the amyloidogenic light chain but also, by co-cultures, their effects on other systems as well as their exerting cellular and organ toxicities. This system will also allow the study of novel agents that may be used and screened as treatments and adjuvant treatments for AL plasma cells.

In another system, assessing the uniqueness of the light chain produced by the amyloid plasma cells, a study showed that amyloid cell lines are particularly dependent upon the balance between the light and heavy chains in the ALMC cell lines (that produce an IgG Lambda intact antibody) [96,97]. A pool of siRNA directed at the light chain constant region reduced light chain production and caused apoptosis in human plasma cells, where the AL cell lines were particularly sensitive to the siRNA. This strategy was tested in a unique murine model utilizing an intraperitoneal xenograft in NOD scid gamma mice employing light chain human myeloma cells [97]. Mice injected intraperitoneally had a robust secretion of the light chain, allowing the future investigation of the small interfering RNA and other treatments for AL in vivo.

This is the beginning of an era of individualized patient therapy, possibly not only to recognize the malignant clone and understand which is the most appropriate therapy but also to be able to recognize the aberrant amyloidogenic light chain and prevent its damage.

In summary, the lessons learnt from patient care support the evidence that the primary amyloidogenic clone is not only unique at the plasma cell level but also that organ damage is not solely due to the deposition of material in the tissues and that the AL light chains precursors are likely to mediate cellular toxicity. The past years have enriched the data coming from everyday practice in the diagnosis and treatment of AL, from the etiological understanding of the basis of the disease to better diagnosis and improved patient care.

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References

1. Kyle, R.A.; Rajkumar, S.V. Epidemiology of the plasma-cell disorders. *Best Pr. Res. Clin. Haematol.* **2007**, *20*, 637–664. [CrossRef]
2. Palumbo, A.; Anderson, K. Multiple myeloma. *N. Engl. J. Med.* **2011**, *364*, 1046–1060. [CrossRef] [PubMed]
3. Korde, N.; Kristinsson, S.Y.; Landgren, O. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM): Novel biological insights and development of early treatment strategies. *Blood* **2011**, *117*, 5573–5581. [CrossRef]
4. Kyle, R.A.; Rajkumar, S.V.; Buadi, F.I. Management of monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM). *Oncology* **2011**, *25*, 578–586.
5. Gertz, M.A. Immunoglobulin light chain amyloidosis: 2013 update on diagnosis, prognosis, and treatment. *Am. J. Hematol.* **2013**, *88*, 416–425. [CrossRef] [PubMed]
6. Merlini, G.; Seldin, D.C.; Gertz, M.A. Amyloidosis: Pathogenesis and New Therapeutic Options. *J. Clin. Oncol.* **2011**, *29*, 1924–1933. [CrossRef] [PubMed]
7. Merlini, G.; Stone, M.J. Dangerous small B-cell clones. *Blood* **2006**, *108*, 2520–2530. [CrossRef] [PubMed]
8. Obici, L.; Perfetti, V.; Palladini, G.; Moratti, R.; Merlini, G. Clinical aspects of systemic amyloid diseases. *Biochim. Biophys. Acta Proteins Proteom.* **2005**, *1753*, 11–22. [CrossRef]
9. Bochtler, T.; Hegenbart, U.; Cremer, F.W.; Heiss, C.; Benner, A.; Hose, D.; Moos, M.; Bila, J.; Bartram, C.R.; Ho, A.D.; et al. Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared with monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability. *Blood* **2008**, *111*, 4700–4705. [CrossRef] [PubMed]
10. Granzow, M.; Hegenbart, U.; Hinderhofer, K.; Hose, D.; Seckinger, A.; Bochtler, T.; Hemminki, K.; Goldschmidt, H.; Schönland, S.O.; Jauch, A. Novel recurrent chromosomal aberrations detected in clonal plasma cells of light chain amyloidosis patients show potential adverse prognostic effect: First results from a genome-wide copy number array analysis. *Haematologica* **2017**, *102*, 1281–1290. [CrossRef] [PubMed]
11. Puig, N.; Paiva, B.; Lasa, M.; Burgos, L.; Perez, J.J.; Merino, J.; Moreno, C.; Vidriales, M.-B.; Toboso, D.G.; Cedena, M.-T.; et al. Flow cytometry for fast screening and automated risk assessment in systemic light-chain amyloidosis. *Leukemia* **2018**, *33*, 1256–1267. [CrossRef]

12. Alameda, D.; Goicoechea, I.; Vicari, M.; Arriazu, E.; Nevone, A.; Rodriguez, S.; Lasa, M.; Puig, N.; Cedena, M.T.; Alignani, D.; et al. Tumor cells in light-chain amyloidosis and myeloma show distinct transcriptional rewiring of normal plasma cell development. *Blood* **2021**, *138*, 1583–1589. [CrossRef]
13. Cuenca, I.; Alameda, D.; Sanchez-Vega, B.; Gómez-Sánchez, D.; Alignani, D.; Lasa, M.; Onecha, E.; Lecumberri, R.; Prosper, F.; Ocio, E.M.; et al. Immunogenetic characterization of clonal plasma cells in systemic light-chain amyloidosis. *Leukemia* **2021**, *35*, 245–249. [CrossRef]
14. Ledergor, G.; Weiner, A.; Zada, M.; Wang, S.-Y.; Cohen, Y.C.; Gatt, M.E.; Snir, N.; Magen, H.; Koren-Michowitz, M.; Herzog-Tzarfati, K.; et al. Single cell dissection of plasma cell heterogeneity in symptomatic and asymptomatic myeloma. *Nat. Med.* **2018**, *24*, 1867–1876. [CrossRef]
15. Merlini, G.; Palladini, G. Differential diagnosis of monoclonal gammopathy of undetermined significance. *Hematology* **2012**, *2012*, 595–603. [CrossRef]
16. Palladini, G.; Merlini, G. What is new in diagnosis and management of light chain amyloidosis? *Blood* **2016**, *128*, 159–168. [CrossRef] [PubMed]
17. Gatt, M.E.; Palladini, G. Light chain amyloidosis 2012: A new era. *Br. J. Haematol.* **2013**, *160*, 582–598. [CrossRef] [PubMed]
18. Lousada, I.; Comenzo, R.L.; Landau, H.; Guthrie, S.; Merlini, G. Light Chain Amyloidosis: Patient Experience Survey from the Amyloidosis Research Consortium. *Adv. Ther.* **2015**, *32*, 920–928. [CrossRef]
19. McCausland, K.L.; White, M.K.; Guthrie, S.D.; Quock, T.; Finkel, M.; Lousada, I.; Bayliss, M.S. Light Chain (AL) Amyloidosis: The Journey to Diagnosis. *Patient Patient Cent. Outcomes Res.* **2018**, *11*, 207–216. [CrossRef] [PubMed]
20. Bayliss, M.; McCausland, K.L.; Guthrie, S.D.; White, M.K. The burden of amyloid light chain amyloidosis on health-related quality of life. *Orphanet J. Rare Dis.* **2017**, *12*, 1–10. [CrossRef]
21. Milani, P.; Valentini, V.; Ferraro, G.; Basset, M.; Russo, F.; Foli, A.; Palladini, G.; Merlini, G. A patient with AL amyloidosis with negative free light chain results. *Clin. Chem. Lab. Med.* **2016**, *54*, 1035–1037. [CrossRef] [PubMed]
22. Kastritis, E.; Papassotiriou, I.; Merlini, G.; Milani, P.; Terpos, E.; Basset, M.; Akalestos, A.; Russo, F.; Psimenou, E.; Apostolakou, F.; et al. Growth differentiation factor-15 is a new biomarker for survival and renal outcomes in light chain amyloidosis. *Blood* **2018**, *131*, 1568–1575. [CrossRef]
23. Kastritis, E.; Papassotiriou, I.; Terpos, E.; Roussou, M.; Gavriatopoulou, M.; Komitopoulou, A.; Skevaki, C.; Eleutherakis-Papaiakovou, E.; Pamboucas, C.; Psimenou, E.; et al. Clinical and prognostic significance of serum levels of von Willebrand factor and ADAMTS-13 antigens in AL amyloidosis. *Blood* **2016**, *128*, 405–409. [CrossRef]
24. Kastritis, E.; Roussou, M.; Michael, M.; Gavriatopoulou, M.; Michalis, E.; Migkou, M.; Delimpasi, S.; Kyrtsonis, M.C.; Gogos, D.; Liapis, K.; et al. High levels of serum angiogenic growth factors in patients with AL amyloidosis: Comparisons with normal individuals and multiple myeloma patients. *Br. J. Haematol.* **2010**, *150*, 587–591. [CrossRef]
25. Muchtar, E.; Dispenzieri, A.; Kumar, S.K.; Buadi, F.K.; Lacy, M.Q.; Zeldenrust, S.; Hayman, S.R.; Leung, N.; Kourelis, T.V.; Gonsalves, W.; et al. Immunoparesis in newly diagnosed AL amyloidosis is a marker for response and survival. *Leukemia* **2016**, *31*, 92–99. [CrossRef] [PubMed]
26. Yogev, D.; Pick, M.; Slyusarevsky, E.; Pogrebijski, G.; Pickin, A.; Gatt, M.E. Serum Hevylite ®assay in the differential diagnosis of patients with high suspicion of AL Amyloidosis. *Int. J. Lab. Hematol.* **2021**, *43*, 418–425. [CrossRef] [PubMed]
27. Bellotti, V.; Chiti, F. Amyloidogenesis in its biological environment: Challenging a fundamental issue in protein misfolding diseases. *Curr. Opin. Struct. Biol.* **2008**, *18*, 771–779. [CrossRef] [PubMed]
28. Kaplan, B.; Golderman, S.; Aizenbud, B.; Esev, K.; Kukuy, O.; Leiba, M.; Livneh, A.; Ben-Zvi, I. Immunoglobulin-free light chain monomer-dimer patterns help to distinguish malignant from premalignant monoclonal gammopathies: A pilot study. *Am. J. Hematol.* **2014**, *89*, 882–888. [CrossRef]
29. Gatt, M.E.; Kaplan, B.; Yogev, D.; Slyusarevsky, E.; Pogrebijski, G.; Golderman, S.; Kukuy, O.; Livneh, A. The use of serum free light chain dimerization patterns assist in the diagnosis of AL amyloidosis. *Br. J. Haematol.* **2018**, *182*, 86–92. [CrossRef]
30. Dispenzieri, A.; Larson, D.R.; Rajkumar, S.V.; Kyle, R.A.; Kumar, S.K.; Kourelis, T.; Arendt, B.; Willrcih, M.; Dasari, S.; Murray, D. N-glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression. *Leukemia* **2020**, *34*, 1–5. [CrossRef]
31. Kourelis, T.; Murray, D.L.; Dasari, S.; Kumar, S.; Barnidge, D.; Madden, B.; Arendt, B.; Milani, P.; Merlini, G.; Ramirez-Alvarado, M.; et al. MASS-FIX may allow identification of patients at risk for light chain amyloidosis before the onset of symptoms. *Am. J. Hematol.* **2018**, *93*, E368–E370. [CrossRef] [PubMed]
32. Mellors, P.W.; Dasari, S.; Kohlhagen, M.C.; Kourelis, T.; Go, R.S.; Muchtar, E.; Gertz, M.A.; Kumar, S.K.; Buadi, F.K.; Willrich, M.A.V.; et al. MASS-FIX for the detection of monoclonal proteins and light chain N-glycosylation in routine clinical practice: A cross-sectional study of 6315 patients. *Blood Cancer J.* **2021**, *11*, 1–9. [CrossRef]
33. Gatt, M.E. Light Chain Amyloidosis 2014. *Int. J. Hematol. Disord.* **2014**, *1*, 21–29.
34. Gatt, M.E.; Hardan, I.; Chubar, E.; Suriu, C.; Tadmor, T.; Shevetz, O.; Patachenco, P.; Dally, N.; Yeganeh, S.; Ballan-Haj, M.; et al. Outcomes of light-chain amyloidosis patients treated with first-line bortezomib: A collaborative retrospective multicenter assessment. *Eur. J. Haematol.* **2015**, *96*, 136–143. [CrossRef]
35. Palladini, G.; Milani, P.; Merlini, G. Management of AL amyloidosis in 2020. *Haematologica* **2020**, *2020*, 363–371. [CrossRef]
36. Sitia, R.; Palladini, G.; Merlini, G. Bortezomib in the treatment of AL amyloidosis: Targeted therapy? *Haematologica* **2007**, *92*, 1302–1307. [CrossRef]

37. Witzig, T.; Timm, M.; Larson, D.; Therneau, T.; Greipp, P. Measurement of apoptosis and proliferation of bone marrow plasma cells in patients with plasma cell proliferative disorders. *Br. J. Haematol.* **1999**, *104*, 131–137. [CrossRef] [PubMed]
38. Oliva, L.; Orfanelli, U.; Resnati, M.; Raimondi, A.; Orsi, A.; Milan, E.; Palladini, G.; Milani, P.; Cerruti, F.; Cascio, P.; et al. The amyloidogenic light chain is a stressor that sensitizes plasma cells to proteasome inhibitor toxicity. *Blood* **2017**, *129*, 2132–2142. [CrossRef]
39. Shragai, T.; Gatt, M.; Lavie, N.; Vaxman, I.; Tadmor, T.; Rouvio, O.; Zektser, M.; Horowitz, N.; Magen, H.; Ballan, M.; et al. Daratumumab for relapsed AL amyloidosis—When cumulative real-world data precedes clinical trials: A multisite study and systematic literature review. *Eur. J. Haematol.* **2021**, *106*, 184–195. [CrossRef]
40. Kimmich, C.R.; Terzer, T.; Benner, A.; Dittrich, T.; Veelken, K.; Carpinteiro, A.; Hansen, T.; Goldschmidt, H.; Seckinger, A.; Hose, D.; et al. Daratumumab for systemic AL amyloidosis: Prognostic factors and adverse outcome with nephrotic-range albuminuria. *Blood* **2020**, *135*, 1517–1530. [CrossRef] [PubMed]
41. Kriegsmann, K.; Dittrich, T.; Neuber, B.; Awwad, M.H.S.; Hegenbart, U.; Goldschmidt, H.; Hillengass, J.; Hose, D.; Seckinger, A.; Müller-Tidow, C.; et al. Quantification of number of CD38 sites on bone marrow plasma cells in patients with light chain amyloidosis and smoldering multiple myeloma. *Cytom. Part B Clin. Cytom.* **2018**, *94*, 767–776. [CrossRef]
42. Seckinger, A.; Hillengass, J.; Emde, M.; Beck, S.; Kimmich, C.; Dittrich, T.; Hundemer, M.; Jauch, A.; Hegenbart, U.; Raab, M.-S.; et al. CD38 as Immunotherapeutic Target in Light Chain Amyloidosis and Multiple Myeloma—Association with Molecular Entities, Risk, Survival, and Mechanisms of Upfront Resistance. *Front. Immunol.* **2018**, *9*, 1676. [CrossRef]
43. Milani, P.; Fazio, F.; Basset, M.; Berno, T.; LaRocca, A.; Foli, A.; Riva, M.; Benigna, F.; Oliva, S.; Nuvolone, M.; et al. High rate of profound clonal and renal responses with daratumumab treatment in heavily pre-treated patients with light chain (AL) amyloidosis and high bone marrow plasma cell infiltrate. *Am. J. Hematol.* **2020**, *95*, 900–905. [CrossRef]
44. Palladini, G.; Milani, P.; Malavasi, F.; Merlini, G. Daratumumab in the Treatment of Light-Chain (AL) Amyloidosis. *Cells* **2021**, *10*, 545. [CrossRef] [PubMed]
45. Kastritis, E.; Palladini, G.; Minnema, M.C.; Wechalekar, A.D.; Jaccard, A.; Lee, H.C.; Sanchorawala, V.; Gibbs, S.; Mollee, P.; Venner, C.P.; et al. Daratumumab-Based Treatment for Immunoglobulin Light-Chain Amyloidosis. *N. Engl. J. Med.* **2021**, *385*, 46–58. [CrossRef]
46. Bochtler, T.; Hegenbart, U.; Kunz, C.; Benner, A.; Kimmich, C.; Seckinger, A.; Hose, D.; Goldschmidt, H.; Granzow, M.; Dreger, P.; et al. Prognostic impact of cytogenetic aberrations in AL amyloidosis patients after high-dose melphalan: A long-term follow-up study. *Blood* **2016**, *128*, 594–602. [CrossRef] [PubMed]
47. Kastritis, E.; Roussou, M.; Gavriatopoulou, M.; Migkou, M.; Kalapanida, D.; Pamboucas, C.; Kaldara, E.; Ntalianis, A.; Psimenou, E.; Toumanidis, S.T.; et al. Long-term outcomes of primary systemic light chain (AL) amyloidosis in patients treated upfront with bortezomib or lenalidomide and the importance of risk adapted strategies. *Am. J. Hematol.* **2015**, *90*, E60–E65. [CrossRef] [PubMed]
48. Gonsalves, W.I.; Buadi, F.K.; Kumar, S.K. Combination therapy incorporating Bcl-2 inhibition with Venetoclax for the treatment of refractory primary plasma cell leukemia with t (11;14). *Eur. J. Haematol.* **2017**, *100*, 215–217. [CrossRef]
49. Kumar, S.; Kaufman, J.L.; Gasparetto, C.; Mikhael, J.; Vij, R.; Pegourie, B.; Benboubker, L.; Facon, T.; Amiot, M.; Moreau, P.; et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. *Blood* **2017**, *130*, 2401–2409. [CrossRef]
50. Kumar, S.; Vij, R.; Kaufman, J.L.; Mikhael, J.; Facon, T.; Pegourie, B.; Benboubker, L.; Gasparetto, C.; Amiot, M.; Moreau, P.; et al. Venetoclax Monotherapy for Relapsed/Refractory Multiple Myeloma: Safety and Efficacy Results from a Phase I Study. *Blood* **2016**, *128*, 488. [CrossRef]
51. Moreau, P.; Harrison, M.S.; Cavo, M.; De La Rubia, J.; Popat, R.; Gasparetto, C.; Hungria, V.T.; Salwender, H.; Suzuki, K.; Kim, I.; et al. Updated Analysis of Bellini, a Phase 3 Study of Venetoclax or Placebo in Combination with Bortezomib and Dexamethasone in Patients with Relapsed/Refractory Multiple Myeloma. *Blood* **2019**, *134*, 1888. [CrossRef]
52. Kaufman, J.L.; Gasparetto, C.; Schjesvold, F.H.; Moreau, P.; Touzeau, C.; Facon, T.; Boise, L.H.; Alzate, S.; Macartney, T.; Pesko, J.; et al. Phase I/II Study Evaluating the Safety and Efficacy of Venetoclax in Combination with Dexamethasone As Targeted Therapy for Patients with t(11;14) Relapsed/Refractory Multiple Myeloma. *Blood* **2019**, *134*, 926. [CrossRef]
53. Le Bras, F.; Dupuis, J.; Lemonnier, F.; Oghina, S.; Bodez, D.; Ladaique, A.; Maarek, A.; Roulin, L.; Ferichou, A.B.; Frenkel, V.; et al. Venetoclax induces sustained complete responses in refractory/relapsed patients with cardiac AL amyloidosis. *J. Clin. Oncol.* **2019**, *37*, e19538. [CrossRef]
54. Leung, N.; Thomé, S.D.; Dispenzieri, A. Venetoclax induced a complete response in a patient with immunoglobulin light chain amyloidosis plateaued on cyclophosphamide, bortezomib and dexamethasone. *Haematologica* **2018**, *103*, e135–e137. [CrossRef] [PubMed]
55. Sidiqi, M.H.; Al Saleh, A.S.; Leung, N.; Aljama, M.; Jevremovic, A.; Gonsalves, W.; Buadi, F.; Kourelis, T.; Warsame, R.; Muchtar, E.; et al. Venetoclax for The Treatment of Translocation AL Amyloidosis. *Clin. Lymphoma Myeloma Leuk.* **2019**, *19*, e332. [CrossRef]
56. Premkumar, V.J.; Lentzsch, S.; Pan, S.; Bhutani, D.; Richter, J.; Jagannath, S.; Liedtke, M.; Jaccard, A.; Wechalekar, A.D.; Comenzo, R.; et al. Venetoclax induces deep hematologic remissions in t(11;14) relapsed/refractory AL amyloidosis. *Blood Cancer J.* **2021**, *11*, 1–10. [CrossRef]
57. D'Souza, A.; Hari, P.; Pasquini, M.; Jacobsen, K.; Flynn, K.E. Baseline patient-reported outcomes in light-chain amyloidosis patients enrolled on an interventional clinical trial. *Amyloid* **2019**, *26*, 87–88. [CrossRef] [PubMed]

58. Sanchorawala, V.; McCausland, K.L.; White, M.K.; Bayliss, M.S.; Guthrie, S.D.; Lo, S.; Skinner, M. A longitudinal evaluation of health-related quality of life in patients with AL amyloidosis: Associations with health outcomes over time. *Br. J. Haematol.* **2017**, *179*, 461–470. [CrossRef]
59. Warsame, R.; Kumar, S.K.; Gertz, M.A.; Lacy, M.Q.; Buadi, F.K.; Hayman, S.R.; Leung, N.; Dingli, D.; Lust, J.A.; Lin, Y.; et al. Hematology patient reported symptom screen to assess quality of life for AL amyloidosis. *Am. J. Hematol.* **2017**, *92*, 435–440. [CrossRef]
60. Jimenez-Zepeda, V.H.; Lee, H.; McCulloch, S.; Tay, J.; Duggan, P.; Neri, P.; Bahlis, N. Treatment response measurements and survival outcomes in a cohort of newly diagnosed AL amyloidosis. *Amyloid* **2021**, *28*, 182–188. [CrossRef] [PubMed]
61. Shen, K.-N.; Miao, H.-L.; Zhang, C.-L.; Feng, J.; Zhang, L.; Cao, X.-X.; Zhou, D.-B.; Wei, S.; Li, J. Posttreatment dFLC less than 10 mg/L predicts superior organ response and longer time to next treatment in newly diagnosed light-chain amyloidosis patients treated with bortezomib. *Leuk. Lymphoma* **2021**, *62*, 874–882. [CrossRef] [PubMed]
62. Palladini, G.; Campana, C.; Klersy, C.; Balduini, A.; Vadacca, G.; Perfetti, V.; Perlini, S.; Obici, L.; Ascari, E.; D'Eril, G.M.; et al. Serum N-Terminal Pro–Brain Natriuretic Peptide is a Sensitive Marker of Myocardial Dysfunction in AL Amyloidosis. *Circulation* **2003**, *107*, 2440–2445. [CrossRef]
63. Palladini, G.; Lavatelli, F.; Russo, P.; Perlini, S.; Perfetti, V.; Bosoni, T.; Obici, L.; Bradwell, A.; D'Eril, G.M.; Fogari, R.; et al. Circulating amyloidogenic free light chains and serum N-terminal natriuretic peptide type B decrease simultaneously in association with improvement of survival in AL. *Blood* **2006**, *107*, 3854–3858. [CrossRef] [PubMed]
64. Kastritis, E.; Kostopoulos, I.V.; Theodorakakou, F.; Fotiou, D.; Gavriatopoulou, M.; Migkou, M.; Tselegkidi, M.I.; Roussou, M.; Papathoma, A.; Eleutherakis-Papaioakovou, E.; et al. Next generation flow cytometry for MRD detection in patients with AL amyloidosis. *Amyloid* **2021**, *28*, 19–23. [CrossRef] [PubMed]
65. Palladini, G.; Paiva, B.; Wechalekar, A.; Massa, M.; Milani, P.; Lasa, M.; Ravichandran, S.; Krsnik, I.; Basset, M.; Burgos, L.; et al. Minimal residual disease negativity by next-generation flow cytometry is associated with improved organ response in AL amyloidosis. *Blood Cancer J.* **2021**, *11*, 1–7. [CrossRef] [PubMed]
66. Merlini, G.; Bellotti, V. Molecular mechanisms of amyloidosis. *N. Engl. J. Med.* **2003**, *349*, 583–596. [CrossRef]
67. Merlini, G.; Westermark, P. The systemic amyloidoses: Clearer understanding of the molecular mechanisms offers hope for more effective therapies. *J. Intern. Med.* **2004**, *255*, 159–178. [CrossRef]
68. Phipps, J.E.; Kestler, D.P.; Foster, J.S.; Kennel, S.J.; Donnell, R.L.; Weiss, D.T.; Solomon, A.; Wall, J. Inhibition of pathologic immunoglobulin-free light chain production by small interfering RNA molecules. *Exp. Hematol.* **2010**, *38*, 1006–1013. [CrossRef]
69. Kourelis, T.V.; Kumar, S.K.; Gertz, M.A.; Lacy, M.Q.; Buadi, F.K.; Hayman, S.R.; Zeldenrust, S.; Leung, N.; Kyle, R.A.; Russell, S.; et al. Coexistent Multiple Myeloma or Increased Bone Marrow Plasma Cells Define Equally High-Risk Populations in Patients with Immunoglobulin Light Chain Amyloidosis. *J. Clin. Oncol.* **2013**, *31*, 4319–4324. [CrossRef]
70. Kumar, S.; Dispenzieri, A.; Lacy, M.Q.; Hayman, S.R.; Buadi, F.K.; Colby, C.; Laumann, K.; Zeldenrust, S.R.; Leung, N.; Dingli, D.; et al. Revised Prognostic Staging System for Light Chain Amyloidosis Incorporating Cardiac Biomarkers and Serum Free Light Chain Measurements. *J. Clin. Oncol.* **2012**, *30*, 989–995. [CrossRef]
71. Rao, M.; Lamont, J.L.; Chan, J.; Concannon, T.W.; Comenzo, R.; Ratichek, S.J.; Avendano, E.E. *Serum Free Light Chain Analysis for the Diagnosis, Management, and Prognosis of Plasma Cell Dyscrasias: Future Research Needs: Identification of Future Research Needs from Comparative Effectiveness Review No. 73*; AHRQ Future Research Needs Papers; Agency for Healthcare Research and Quality: Rockville, MD, USA, 2012.
72. Kumar, S.; Dispenzieri, A.; Katzmann, J.A.; Larson, D.R.; Colby, C.L.; Lacy, M.Q.; Hayman, S.R.; Buadi, F.K.; Leung, N.; Zeldenrust, S.R.; et al. Serum immunoglobulin free light-chain measurement in primary amyloidosis: Prognostic value and correlations with clinical features. *Blood* **2010**, *116*, 5126–5129. [CrossRef]
73. Snozek, C.L.H.; Katzmann, A.J.; Kyle, A.R.; Dispenzieri, A.; Larson, D.R.; Therneau, T.M.; Melton, L.J.; Kumar, S.; Greipp, P.R.; Clark, R.J.; et al. Prognostic value of the serum free light chain ratio in newly diagnosed myeloma: Proposed incorporation into the international staging system. *Leukemia* **2008**, *22*, 1933–1937. [CrossRef]
74. Jenner, E. Serum free light chains in clinical laboratory diagnostics. *Clin. Chim. Acta* **2014**, *427*, 15–20. [CrossRef] [PubMed]
75. Blancas-Mejia, L.M.; Misra, P.; Dick, C.J.; Cooper, S.A.; Redhage, K.R.; Bergman, M.R.; Jordan, T.L.; Maar, K.; Ramirez-Alvarado, M. Immunoglobulin light chain amyloid aggregation. *Chem. Commun.* **2018**, *54*, 10664–10674. [CrossRef] [PubMed]
76. Jordan, T.L.; Maar, K.; Redhage, K.R.; Misra, P.; Blancas-Mejia, L.M.; Dick, C.J.; Wall, J.; Williams, A.; Dietz, A.B.; van Wijnen, A.J.; et al. Light chain amyloidosis induced inflammatory changes in cardiomyocytes and adipose-derived mesenchymal stromal cells. *Leukemia* **2020**, *34*, 1383–1393. [CrossRef]
77. Weber, B.; Hora, M.; Kazman, P.; Pradhan, T.; Rührnößl, F.; Reif, B.; Buchner, J. Domain Interactions Determine the Amyloidogenicity of Antibody Light Chain Mutants. *J. Mol. Biol.* **2020**, *432*, 6187–6199. [CrossRef] [PubMed]
78. Sidana, S.; Tandon, N.; Gertz, M.A.; Dispenzieri, A.; Ramirez-Alvarado, M.; Murray, D.L.; Kourelis, T.V.; Buadi, F.K.; Kapoor, P.; Gonsalves, W.; et al. Clinical features, laboratory characteristics and outcomes of patients with renal versus cardiac light chain amyloidosis. *Br. J. Haematol.* **2019**, *185*, 701–707. [CrossRef] [PubMed]
79. Oberti, L.; Maritan, M.; Rognoni, P.; Barbiroli, A.; Lavatelli, F.; Russo, R.; Palladini, G.; Bolognesi, M.; Merlini, G.; Ricagno, S. The concurrency of several biophysical traits links immunoglobulin light chains with toxicity in AL amyloidosis. *Amyloid* **2019**, *26*, 107–108. [CrossRef]

80. Comenzo, R.L.; Zhang, Y.; Martinez, C.; Osman, K.; Herrera, G.A. The tropism of organ involvement in primary systemic amyloidosis: Contributions of Ig V(L) germ line gene use and clonal plasma cell burden. *Blood* **2001**, *98*, 714–720. [CrossRef]
81. Chen, E.C.; Rubinstein, S.; Soto, C.; Bombardi, R.G.; Day, S.B.; Myers, L.; Zaytsev, A.; Majedi, M.; Cornell, R.F.; Crowe, J.; et al. Diverse patterns of antibody variable gene repertoire disruption in patients with amyloid light chain (AL) amyloidosis. *PLoS ONE* **2020**, *15*, e0235713. [CrossRef] [PubMed]
82. Garofalo, M.; Piccoli, L.; Romeo, M.; Barzago, M.M.; Ravasio, S.; Foglierini, M.; Matkovic, M.; Sgrignani, J.; De Gasparo, R.; Prunotto, M.; et al. Machine learning analyses of antibody somatic mutations predict immunoglobulin light chain toxicity. *Nat. Commun.* **2021**, *12*, 1–10. [CrossRef]
83. Abraham, R.S.; Geyer, S.M.; Price-Troska, T.L.; Allmer, C.; Kyle, R.A.; Gertz, M.A.; Fonseca, R. Immunoglobulin light chain variable (V) region genes influence clinical presentation and outcome in light chain–associated amyloidosis (AL). *Blood* **2003**, *101*, 3801–3807. [CrossRef]
84. Baden, E.M.; Sikkink, L.; Ramirez-Alvarado, M. Light Chain Amyloidosis—Current Findings and Future Prospects. *Curr. Protein Pept. Sci.* **2009**, *10*, 500–508. [CrossRef]
85. Perfetti, V.; Casarini, S.; Palladini, G.; Vignarelli, M.C.; Klersy, C.; Diegoli, M.; Ascari, E.; Merlini, G. Analysis of Vλ-Jλ expression in plasma cells from primary (AL) amyloidosis and normal bone marrow identifies 3r(λIII) as a new amyloid-associated germline gene segment. *Blood* **2002**, *100*, 948–953. [CrossRef] [PubMed]
86. Arendt, B.K.; Ramirez-Alvarado, M.; Sikkink, L.A.; Keats, J.J.; Ahmann, G.J.; Dispenzieri, A.; Fonseca, R.; Ketterling, R.P.; Knudson, R.A.; Mulvihill, E.M.; et al. Biologic and genetic characterization of the novel amyloidogenic lambda light chain–secreting human cell lines, ALMC-1 and ALMC-2. *Blood* **2008**, *112*, 1931–1941. [CrossRef]
87. Lavatelli, F.; Perlman, D.H.; Spencer, B.; Prokaeva, T.; McComb, M.E.; Théberge, R.; Connors, L.; Bellotti, V.; Seldin, D.C.; Merlini, G.; et al. Amyloidogenic and Associated Proteins in Systemic Amyloidosis Proteome of Adipose Tissue. *Mol. Cell. Proteom.* **2008**, *7*, 1570–1583. [CrossRef] [PubMed]
88. Kourelis, T.V.; Dasari, S.S.; Dispenzieri, A.; Maleszewski, J.J.; Redfield, M.M.; Fayyaz, A.U.; Grogan, M.; Ramirez-Alvarado, M.; Ezzeddine, O.F.A.; McPhail, E.D. A Proteomic Atlas of Cardiac Amyloid Plaques. *JACC Cardio Oncol.* **2020**, *2*, 632–643. [CrossRef] [PubMed]
89. Lavatelli, F.; Imperiini, E.; Orrù, S.; Rognoni, P.; Sarnataro, D.; Palladini, G.; Malpasso, G.; Soriano, M.E.; Di Fonzo, A.; Valentini, V.; et al. Novel mitochondrial protein interactors of immunoglobulin light chains causing heart amyloidosis. *FASEB J.* **2015**, *29*, 4614–4628. [CrossRef]
90. Imperlini, E.; Gnecchi, M.; Rognoni, P.; Sabidó, E.; Ciuffreda, M.C.; Palladini, G.; Espadas, G.; Mancuso, F.; Bozzola, M.; Malpasso, G.; et al. Proteotoxicity in cardiac amyloidosis: Amyloidogenic light chains affect the levels of intracellular proteins in human heart cells. *Sci. Rep.* **2017**, *7*, 15661. [CrossRef] [PubMed]
91. Diomede, L.; Rognoni, P.; Lavatelli, F.; Romeo, M.; Di Fonzo, A.; Foray, C.; Fiordaliso, F.; Palladini, G.; Valentini, V.; Perfetti, V.; et al. Investigating heart-specific toxicity of amyloidogenic immunoglobulin light chains: A lesson fromC. elegans. *Worm* **2014**, *3*, e965590. [CrossRef] [PubMed]
92. Mishra, S.; Joshi, S.; Ward, J.E.; Buys, E.P.; Mishra, D.; Morgado, I.; Fisch, S.; Lavatelli, F.; Merlini, G.; Dorbala, S.; et al. Zebrafish model of amyloid light chain cardiotoxicity: Regeneration versus degeneration. *Am. J. Physiol. Circ. Physiol.* **2019**, *316*, H1158–H1166. [CrossRef] [PubMed]
93. Venner, C.P.; Lane, T.; Foard, D.; Rannigan, L.; Gibbs, S.D.J.; Pinney, J.H.; Whelan, C.J.; Lachmann, H.; Gillmore, J.D.; Hawkins, P.N.; et al. Cyclophosphamide, bortezomib, and dexamethasone therapy in AL amyloidosis is associated with high clonal response rates and prolonged progression-free survival. *Blood* **2012**, *119*, 4387–4390. [CrossRef] [PubMed]
94. Saunders, C.N.; Chattopadhyay, S.; Huhn, S.; Weinhold, N.; Hoffmann, P.; Nöthen, M.M.; Jöckel, K.-H.; Schmidt, B.; Landi, S.; Goldschmidt, H.; et al. Search for AL amyloidosis risk factors using Mendelian randomization. *Blood Adv.* **2021**, *5*, 2725–2731. [CrossRef]
95. Rius, B.; Mesgarzadeh, J.S.; Romine, I.C.; Paxman, R.J.; Kelly, J.W.; Wiseman, R.L. Pharmacologic targeting of plasma cell endoplasmic reticulum proteostasis to reduce amyloidogenic light chain secretion. *Blood Adv.* **2021**, *5*, 1037–1049. [CrossRef]
96. Zhou, P.; Ma, X.; Iyer, L.; Chaulagain, C.; Comenzo, R.L. One siRNA pool targeting the λ constant region stops λ light-chain production and causes terminal endoplasmic reticulum stress. *Blood* **2014**, *123*, 3440–3451. [CrossRef] [PubMed]
97. Ma, X.; Zhou, P.; Kugelmass, A.; Toskic, D.; Warner, M.; Lee, L.; Fogaren, T.; Godara, A.; Wang, M.; Li, Y.; et al. A novel xenograft mouse model for testing approaches targeting human kappa light-chain diseases. *Gene Ther.* **2019**, *26*, 187–197. [CrossRef]