

Review

Zilucoplan: A Newly Approved Macrocyclic Peptide for Treatment of Anti-Acetylcholine Receptor Positive Myasthenia Gravis

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Abstract: Zilucoplan is a synthetic macrocyclic peptide approved by the Food and Drug Administration (FDA), in October 2023, for the treatment of generalized myasthenia gravis. It is considered as an orphan drug that causes the inhibition of terminal complement cascade activation with a dual mechanism of action preventing the formation of the membrane attack complex (MAC) and the destruction of the neuromuscular junction. This drug has been demonstrated to be able to treat the generalized myasthenia gravis without significant adverse effects, with good efficacy, safety, and tolerability profile. Zilucoplan is not only innovative and promising in the therapeutics of generalized myasthenia gravis, but it could also be beneficial for the treatment of other diseases as well as a model for synthesis of analogues to improve pharmacological profile.

Keywords: zilucoplan; cyclic peptide; complement component 5 inhibitor; generalized myasthenia gravis; FDA approved



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1. Introduction

Peptides are at the top of current research for new selective, potent, and safe therapeutic agents [1,2]. Peptides are a unique class of molecules composed of two or more residues of amino acids with structurally diverse chemical compositions, sizes, and shapes. They can be considered between small molecules and large biological molecules such as proteins [3].

Over the last decades, this class of compounds have been captivated great attention in different fields, including pharmaceutical [4–6], nutraceutical [7,8], and cosmeceutical [9,10] industries. Another relevant application of peptides is in drug delivery considering the capability of these molecules to organize themselves to form nanostructures. Peptide nanostructures proved to have ability to form stable drug-complexes, displaying high drug load capacity and protection [11,12]. In addition, cell penetrating peptides demonstrated to be a powerful tool for targeted drug delivery [13]. Peptides are also used as imaging and disease diagnosis tools [14] as well as chiral stationary phases for the chromatographic enantioseparation of chiral analytes, due to their high capacity for chiral recognition [15,16].

Until December 2023, 119 peptides had been approved by the competent regulatory authorities as new drugs and, among them, around 46% are cyclic peptides [17]. Gramicidin S (antibiotic), discovered during the Second World War, was the first cyclic peptide to be used in therapeutics [18,19]. Other remarkable examples of cyclic peptides as drugs are vancomycin, telavancin, dalbavancin, and oritavancin (antibiotics) [20], anidulafungin and rezafungin (antifungals) [21,22], lanreotide, pasireotide, and romidepsin (anticancer drugs) [23], and linaclotide (for gastrointestinal (GI) disorders) [24].

The newly approved cyclic peptide was zilucoplan (Figure 1), a macrocyclic peptide used for treatment of generalized myasthenia gravis [25,26].

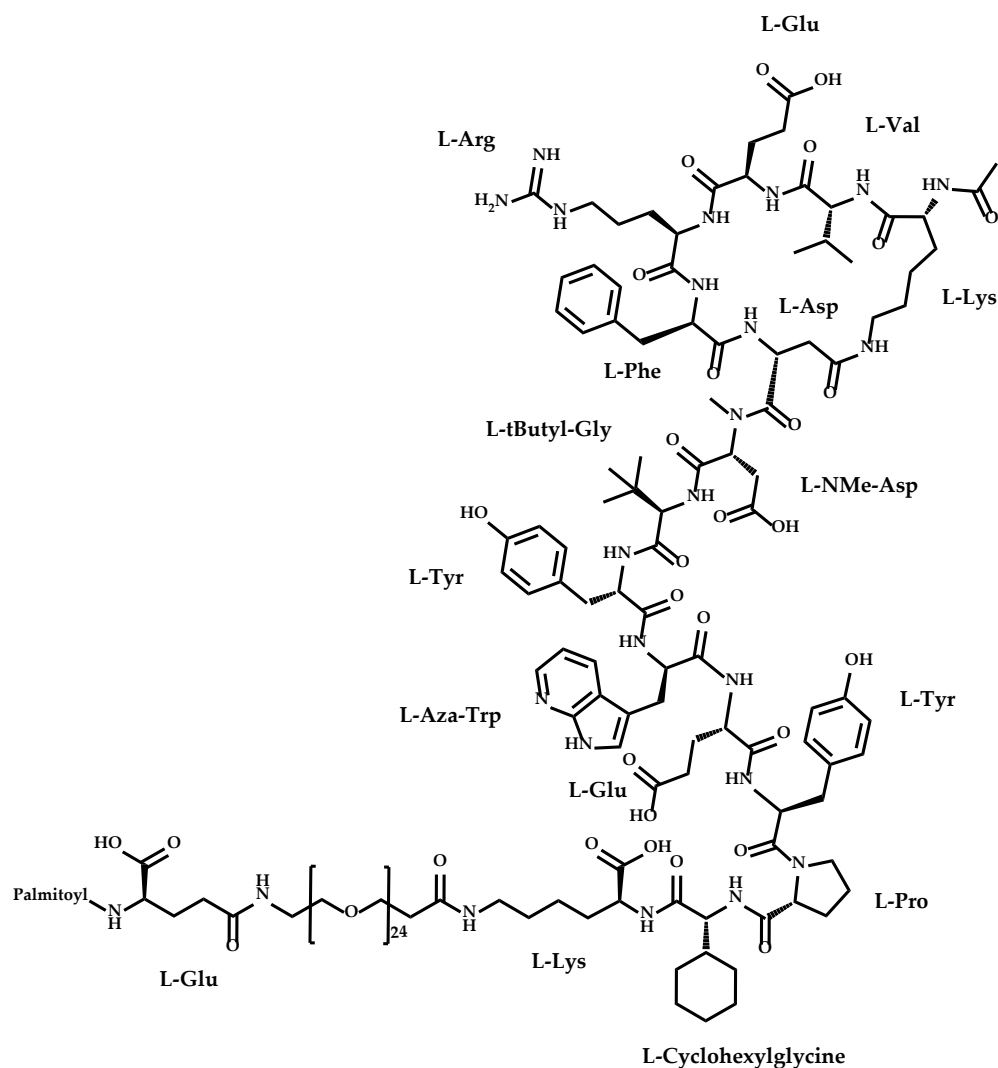


Figure 1. Structure of zilucoplan.

Zilucoplan (Table 1), also designated as RA101495, is the active principle of Zilbrysq[®], commercialized by UCB Pharma S.A. It is a 3.5 kDa synthetic macrocyclic peptide composed of 15 amino acid residues, including four unnatural amino acids [27]. The amino acid residues composition is: L-Lys, L-Val, L-Glu, L-Arg, L-Phe, L-Asp, L-L-NMe-Asp, L-tButyl-Gly, L-Tyr, L-7-aza-Trp, L-Glu, L-Tyr, L-Pro, L-Cyclohexyl-Gly, and L-Lys.

Polyethylene glycol 24, γ -glutamic acid and an *N*-terminal palmitic acid moiety were attached to the side chain of a L-Lys residue (Figure 1) [28]. The addition of a C16 lipid via a short monodisperse polyethylene glycol linker provides a pharmacokinetic profile consistent with daily dosing in humans [29].

Zilucoplan was developed through a targeted screen using the innovative extreme diversity mRNA display platform [30–33]. This drug received the designation of orphan drug for the treatment of myasthenia gravis in the European Union in July 2018 from the European Medicines Agency (EMA) [34]. The EMA defines an orphan drug as a drug that is used for the diagnosis, prevention, or treatment of a life-threatening or chronically/seriously debilitating condition that affects no more than 5 in 10,000 people in the European Union [35].

The aim and novelty of this review is to compile all the specific information about zilucoplan.

the cell by passive diffusion or active transport. Cyclization also affords better metabolic stability, because cyclic peptides are resistant to the action of both exopeptidases and endopeptidases [14,60,64]. In addition to cyclization, there are also disulfide bonds which play a very important role in peptides as they allow constrained and defined structures to be created [65]. Thus, it is possible to further enhance the structural and metabolic stability, selectivity, potency and permeability of the peptides, since the structure becomes even more rigid than it would be with cyclization exclusively [66]. The full potential and advantages of cyclic peptides containing disulfide bonds have already been explored in some scientific articles [65,66].

Cyclization is the most challenging step to obtain cyclic peptides; therefore, some strategies have been developed to facilitate this process [38]. In addition to the classical cyclization process in solution, using coupling reagents and protecting groups [67,68], new methods of cyclization have emerged, including native chemical ligation [69], serine/threonine ligation [70], KAHA ligation [71], Staudinger's ligation [72,73], enzyme-mediated cyclization [74], Cyclick strategy [75], on-resin cyclization [41,76], among others. These strategies ensure that the difficulties inherent to cyclization in solution can be overcome, namely the possibility of C-terminal epimerization, oligomerization, and low coupling efficiency according to the ring size and linear peptide sequence. In addition, the full protection of the side chains is not required and a chemoselective bond between two amino acid residues is obtained, allowing an increase of the efficiency of cyclization [67,68]. However, it is important to highlight that some of these strategies are still not widely used, since the presence of certain functional groups on the amino acid residues is mandatory for cyclization to take place, which can be unfeasible for obtaining a desired cyclic peptide structure [64].

The rewards of cyclization, combined with the intrinsic and unique characteristics of peptides, make cyclic peptides very attractive molecules for the development of new drugs with diverse biological activities [64,77]. Most of the cyclic peptides were explored as antimicrobial and anticancer agents [78–81].

2.2. Reported Procedures

Zilucoplan has been obtained by total synthesis [28,82]. As mentioned before, chemical synthesis is an important source of peptides for drug discovery and development. Numerous advantages characterize the synthetic strategy, including the possibility of obtaining high quantities of peptides required for large-scale biological assays and/or drug development. Another noteworthy benefit of synthesis is to allow the preparation of peptides comprising the desired structure as well as to achieve structurally diverse analogues and derivatives for structure–activity relationship studies [40–42,83].

A brief description of the synthesis of zilucoplan is reported in patent literature [82,84–86] and in an original article by Gorman et al. [28]. Zilucoplan was synthesized in three steps (Figure 2). First, a linear peptide sequence was synthesized by coupling amino acid residues [82]. In general, peptide synthesis can be carried out in solution, in solid phase, or by using these two strategies simultaneously [79]. Both include two key steps for the formation of a peptide bond between two amino acid residues: the activation of the carboxyl group by coupling reagents and the temporary use of protecting groups to direct the reaction to the desired direction [87]. The two main strategies are fluorenylmethyloxycarbonyl (Fmoc)/*tert*-butyl (tBu) and *tert*-butyloxycarbonyl (Boc)/benzyl (Bn) [88].

For zilucoplan, this step was carried out using a standard solid-phase Fmoc/tBu method. The synthesis was performed in a Liberty automated microwave peptide synthesizer following standard protocols with Rink amide resin. The coupling agent used was 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate (HCTU) with diisopropyl ethylamine (DIEA) as catalytic base [82].

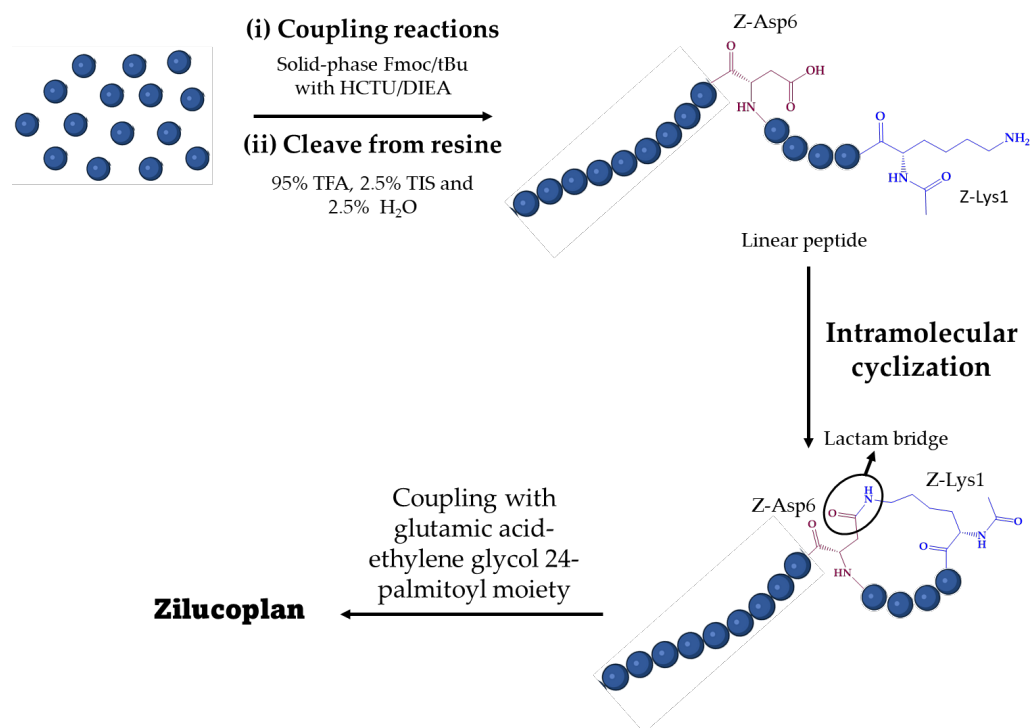


Figure 2. General synthetic pathway for zilucoplan. HCTU: 2-(6-chloro-1-*H*-benzotriazole-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate; DIEA: diisopropyl ethylamine; Fmoc: fluorenylmethyloxycarbonyl; tBu: *tert*-butyl; TFA: trifluoroacetic acid; TIS: triisopropylsilane.

After this process, the polypeptide chain was cleaved from the resin and side chain-protecting groups were removed by treatment with 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS) and 2.5% water for 3 h at room temperature and was isolated by precipitation with ether [82]. The crude linear polypeptide was purified by preparative reverse phase high-performance liquid chromatography (RP-HPLC) using a C18 column [82]. The pure linear peptide was analyzed by liquid chromatography-mass spectrometry (LC-MS) (Figure 3). RP-HPLC was performed with an increasing gradient of 1% buffer B (90% acetonitrile, 0.05% TFA) in buffer A (0.05% TFA) per minute [28].

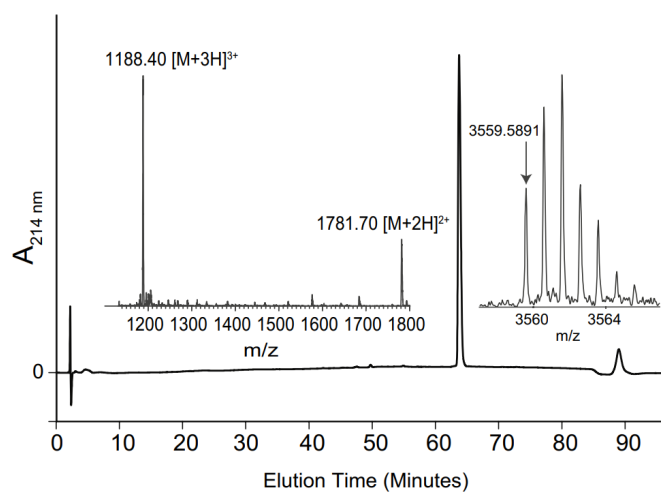


Figure 3. RP-HPLC chromatogram, electrospray ionization mass spectrometry (ESI-MS) spectrum and MALDI-MS spectrum of zilucoplan [28].

The next synthetic step was the intramolecular cyclization of the linear peptide [82]. Cyclization of a peptide can occur in four different ways, depending on the amino acid residues present: head-to-tail cyclization, head-to-side chain cyclization, side-chain-to-tail cyclization, and side-chain-to-side-chain cyclization [89]. For zilucoplan, a head-to-side chain cyclization was carried out via a lactam bridge between the side chains of the Z-Lys1 and Z-Asp6 residues. Finally, a glutamic acid-ethylene glycol24-palmitoyl moiety was coupled to a second Lys sidechain affording the zilucoplan [82].

The planar structure of zilucoplan was established by comprehensive analyses of two-Dimensional ^1H - ^1H TOCSY and NOESY experiments, and the secondary structure was identified via the determination of secondary $\text{H}\alpha$ shifts from the closest naturally occurring residues random coil values [28].

3. Zilucoplan and Myasthenia Gravis

3.1. The Disease

Myasthenia gravis is a chronic, unpredictable, and antibody-mediated organ specific autoimmune disease commonly associated with thymic changes [90,91]. Systematic epidemiological studies revealed that its annual incidence is estimated to range from 7–23 cases per million, while the prevalence varies from 70–320 cases per million [92]. Nevertheless, although the incidence of this disease may vary according to the geographical region in question, its prevalence has been increasing over the last seven decades [93]. This trend may be explained by the evolution of knowledge about the disease and the development of diagnostic methods [94]. Nevertheless, the mortality rate has decreased significantly since the introduction of various treatment options [93].

This debilitating rare disease is associated with loss of muscle strength and is worsened by physical exercise or repeated use of the muscles [95,96]. This disease first manifests itself with eye symptoms such as ptosis, diplopia (double vision), or blurred vision [97]. Normally, around 80% of patients with these symptoms progress to generalized myasthenia gravis with generalized symptoms in two or three years [98–100]. These generalized symptoms are related to bulbar manifestations like dysphagia, weakness and fatigue [97]. In more than 40% of patients, there is muscular respiratory weakness that causes dyspnoea on exertion or orthopnoea [100]. Around 15% to 20% of patients will suffer a myasthenic crisis, which results in respiratory failure, requiring ventilation [97,100]. Prolonged muscle weakness can lead to obesity and respiratory infections. Other comorbidities of this disease can be the appearance of other autoimmune diseases and thymic malignancies [101].

The damage caused by this disease is due to autoantibodies targeting certain components of the neuromuscular junction (NMJ) [102]. Depending on the components targeted by the autoantibodies, this disease is subdivided into different subtypes: the most common subtype is characterized by autoantibodies targeting nicotinic acetylcholine receptors (AChR), this is the subtype that affects around 85% of patients; there is also the case where autoantibodies target muscle-specific kinase (MuSK) or lipoprotein receptor-related protein 4 (LRP4) these situations affect around 15% of patients [103–106]. There is also a more unusual situation in which circulating autoantibodies to known targets are not detected; this only happens in a small portion of patients [91].

AChR MG autoantibodies belong to subtypes that mediate tissue damage at the NMJ, while MuSK MG autoantibodies belong to subtypes that, by interfering with protein–protein interactions at the NMJ, cause direct disruption of AChR signaling [107,108]. When diagnosing the disease, it is extremely important to identify the subtype of the disease, since each subtype has different immunopathologies and the treatments required will therefore be different [91]. This review will only explore MG caused by autoantibodies targeting AChR.

Three pathogenic mechanisms of AChR auto-antibodies have already been identified: Firstly, there is the mechanism in which AChR auto-antibodies inhibit the binding of acetylcholine (ACh) to its receptors by binding to the active site of the receptor or by binding close to the active site. This will result in a decrease in ACh-dependent signaling at

the NMJ [109]. The second mechanism consists of auto-antibody-mediated crosslinking, which results in the internalization of the AChR, decreasing the number of these receptors at the NMJ. This mechanism is known as antigen modulation [110,111]. The third mechanism is related to the immunoglobulin effector functions of AChR auto-antibodies. One of these main functions is the activation of the complement cascade, which leads to the formation of the membrane attack complex (MAC) and causes damage to the postsynaptic membrane and the destruction of synaptic components [112]. The latter is the predominant mechanism and will be explored below [93].

In this mechanism, AChR autoantibodies ACh receptors located in the post-synaptic membrane of neuromuscular junctions, which significantly reduces the number of these receptors. This attack triggers the activation of the antibody-dependent complement system which leads to the formation of MAC. The accumulation of MAC in the post-synaptic membrane of neuromuscular junctions is responsible for their destruction, which leads to muscle weakness (Figure 4A) [108].

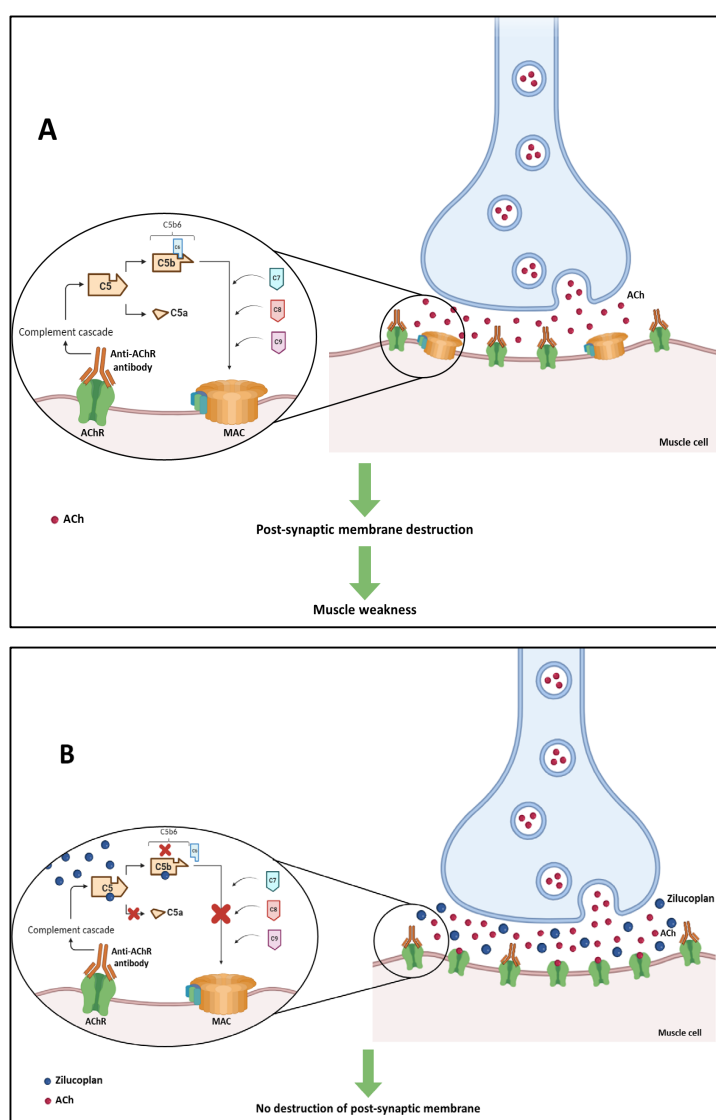


Figure 4. (A) Activation of the antibody-dependent complement system in generalized myasthenia gravis by attack of anti-acetylcholine receptor (anti-AChR) antibodies, leading to the destruction of the post-synaptic membrane of neuromuscular junctions. (B) Mechanism of action of zilucoplan blocking the complement cascade and preventing the formation of membrane attack complex (MAC) and the destruction of the neuromuscular junction.

Initially, scarce information was available about this disease or its physiological causes and, as a result, the prognosis for patients was very serious and the mortality rate was high. However, as scientific knowledge progressed, the causes and mechanism of this disease were discovered and, as a consequence, treatments began to be developed [113]. In recent decades, there has been great progress in the treatment of myasthenia gravis [90], being considered one of the most treatable autoimmune diseases [114].

3.2. Therapeutic Strategies

Currently, therapeutic strategies for myasthenia gravis disease are divided into two main groups [108]. One group includes traditional therapies such as the use of non-immunosuppressants such as pyridostigmine, which is an acetylcholinesterase (AChE) inhibitor that increases the amount of ACh in the synapses [115]. Among the traditional therapies there are also immunosuppressants such as corticosteroids, calcineurin inhibitors (cyclosporine A, tacrolimus), antimetabolites (cyclophosphamide, mycophenolate mofetil and methotrexate), and intravenous immunoglobulin (IVIG) therapy, with a diverse range of mechanisms of action [108,116]. Plasma exchange (PLEX) is another therapeutic approach, consisting of the direct removal of autoantibodies and complement components involved in the pathogenesis of the disease, in addition with the decrease of pro-inflammatory cytokines levels [117–119]. The traditional therapies are used to control the symptoms of muscle weakness [27].

On the other hand, there are therapies that use biological molecules that exert therapeutic effects in less time and have fewer side effects, when compared with traditional therapies, because its mechanism of action is more targeted [114]. Therapies using biologicals include B-cell-targeted drugs (rituximab, obinutuzumab, belimumab and inebilizumab), T-cell-targeted drugs (iscalimab, tocilizumab), complement inhibitors (eculizumab and ravulizumab), proteasome inhibitors (bortezomib and mezagitamab), and neonatal Fc receptor antibody therapy (efgartigimod, rozanolixizumab, nipocalimab and batoclimab) [108,120]. The development of biologicals can be considered as a turning point in the treatment of myasthenia gravis [114]. However, all these options have numerous side effects that need to be constantly monitored. In the case of the use of complement system inhibitor antibodies (like eculizumab and ravulizumab) it is even more complicated since these are associated with opportunistic infections like meningococcal infection, and can trigger immunogenicity problems that can reduce the effectiveness of the treatment [108,121]. These hurdles can be overcome with a non-antibody-based complement inhibition strategy like zilucoplan [121]. Zilucoplan, a newly approved drug, emerges as an innovative and promising solution for the treatment of this disease.

3.3. Zilucoplan

3.3.1. Mechanism of Action

Zilucoplan is an inhibitor of terminal complement cascade activation, which is initiated by anti-AChR antibodies. This drug has a dual mechanism of action: it binds to complement 5 (C5) with high affinity and specificity, preventing the conversion of this complement into C5a and C5b by C5 convertase, and it binds to the previously formed C5b moiety, sterically preventing its interaction with C6 (Figure 4B) [29]. Both C5a and C5b metabolites are associated with host defense, with C5a propagating leukocyte chemotaxis and cytokine release through its receptors C5aR1 and C5aR2 [122], and C5b initiating the formation of MAC.

The dual mechanism of zilucoplan prevents the assembly and activity of MAC. Since the MAC complex is not formed, there is no destruction of the postsynaptic membrane [29].

3.3.2. Indication

In September 2023, the Committee for Medicinal Products for Human Use (CHMP) of the EMA issued a positive opinion recommending the granting of a marketing authorization for Zilbrysq[®], intended for the treatment of myasthenia gravis [123]. On 25 September

2023, zilucoplan was first approved by the Japanese Ministry of Health, Labour and Welfare (MHLW) for the treatment of adults with generalized myasthenia gravis in adults who are AChR antibody-positive and inappropriately respond to steroids or other immunosuppressants [124]. Then, it was approved in the USA, on 17 October 2023, [25,125,126] and in the EU, on 4 December 2023, for the treatment of generalized myasthenia gravis in adult patients who are anti-AChR antibody positive [127].

It is administered via subcutaneous self-injection once a day [128]. This is an advantage over the other C5 inhibitors that are currently available since they are administered intravenously. For patients, self-administered subcutaneous treatment is more convenient because it prevents the need for regular clinic visits, and consequently, improves patients' autonomy and reduces daily life interference. In addition, if rescue therapy is required, the treatment with a peptide C5 inhibitor has the additional benefit of allowing the simultaneous use of plasma exchange and intravenous immunoglobulin, without need for supplemental dosing [27].

3.3.3. Clinical Trials

This drug completed several clinical trials before it was approved, which are summarized in Figure 5.

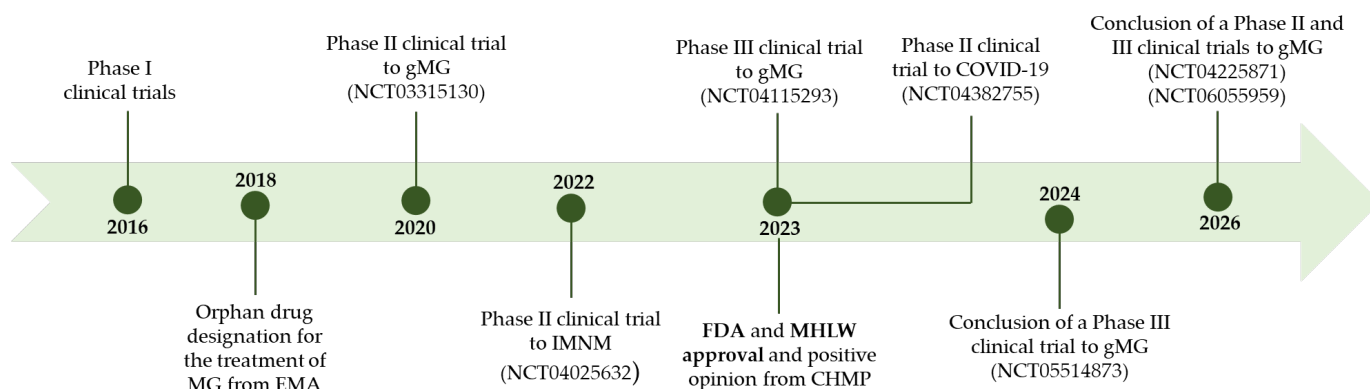


Figure 5. Pipeline of zilucoplan. gMG: generalized myasthenia gravis.

Phase I clinical trials were carried out to assess the pharmacokinetic and pharmacodynamic properties of zilucoplan and its safety and tolerability after subcutaneous injection [129,130]. The first study was a Phase I, randomized, double-blind, placebo-controlled, single-escalating-dose study in which single doses of zilucoplan (0.05 mg/kg to 0.4 mg/kg) were administered [129]. This trial involved 22 healthy volunteers. From a pharmacokinetic point of view, this study has shown that zilucoplan plasma levels are consistent with the values predicted by *in silico* pharmacokinetic models. Furthermore, the relationship between the maximum concentration in plasma and the dose administered was linear, demonstrating dose-dependent exposure. At all doses administered, the $t_{1/2}$ was approximately 7 days [129].

With regard to pharmacodynamics, this study showed that zilucoplan has a rapid dose-dependent inhibition of haemolysis and suppression of complement activity [129].

Furthermore, this study concluded that zilucoplan is apparently safe and well tolerated. This preliminary trial suggested that with low-dose administration, steady-state levels of haemolysis suppression of over 90% can be achieved [129].

The second Phase I trial was a randomized, double-blind, placebo-controlled, multiple-dose study, in which multiple doses of zilucoplan (0.2 mg/kg) were administered over 7 days [130]. This trial involved six healthy volunteers. Regarding pharmacokinetics, it was possible to conclude that the drug levels achieved were consistent with the prediction made in an *in silico* pharmacokinetic model and were in agreement with the values observed in the previous Phase I trial [129,130]. Plasma levels of zilucoplan showed low variability in

exposure in all subjects and peak drug levels were observed after 3 h in all participants. In addition, the results obtained allowed the prediction that with the administration of a dose of 0.2 mg/kg, zilucoplan plasma concentrations reach steady-state on day 11 [130]. From a pharmacodynamic point of view, it was concluded that the inhibition of haemolysis was rapid and sustained in 95% with the administration of daily doses of 0.2 mg/kg for 7 days. As for the suppression of complement activity, at the dose and for the period tested this drug demonstrated the capacity for rapid, complete, and sustained suppression. Furthermore, this trial showed that zilucoplan is safe and well tolerated for 7 days at the dose tested [130].

A Phase II clinical trial (NCT03315130) was carried out to evaluate the clinical effects of zilucoplan when administered via subcutaneous injection to adult patients with moderate to severe generalized myasthenia gravis [128]. This was a randomized, double-blind, placebo-controlled, multicenter clinical trial in which 44 patients with generalized myasthenia gravis took part. During the study, the patients maintained the therapy that they were using to treat generalized myasthenia gravis [128].

In this study, doses of 0.1 mg/kg and 0.3 mg/kg were tested and injected daily for 12 weeks. The 0.3 mg/kg dose was the one that showed the most rapid improvement in the disease. With this study it was possible to conclude that zilucoplan administered at a dose of 0.3 mg/kg is an effective complement inhibitor for the majority of patients with moderate to severe generalized myasthenia gravis. Moreover, safety and tolerability profiles were favourable [128].

After these 12 weeks, the patients who took part in this Phase II trial were eligible to be enrolled in an open-label extension period with the aim of confirming the dose to be used in a Phase III trial. Once again, zilucoplan showed a good safety and tolerability profile for both doses and the dose that allowed for the most significant results in the shortest period of time was 0.3 mg/kg; thus, this was the chosen dose [128,131].

A Phase III trial (RAISE) (NCT04115293) evaluated the safety and efficacy of zilucoplan when administered to patients with AChR autoantibody positive generalized myasthenia gravis [132]. RAISE was a randomized, double-blind, and placebo-controlled trial. This trial was carried out in Europe, Japan, and North America and involved 174 patients with AChR autoantibody positive generalized myasthenia gravis. In this study, zilucoplan was administered once a day at a dose of 0.3 mg/kg by subcutaneous self-injection. Zilucoplan demonstrated rapid and significant improvements in the treatment of generalized myasthenia gravis and proved to be effective. In this trial, similar percentages of patients in the zilucoplan and placebo groups experienced any-grade treatment-emergent adverse events (TEAEs) (77 vs. 70%), serious TEAEs (13 vs. 15%) and severe TEAEs (12 vs. 13%). The most frequently described TEAEs in the zilucoplan and placebo groups were injection-site pain (9 vs. 3%), injection-site bruising (16 vs. 9%), headache (15 vs. 16%), diarrhoea (10 vs. 2%), and myasthenia gravis worsening (10 vs. 9%). In the zilucoplan and placebo groups, 5% and 2% of patients, respectively, discontinued treatment due to TEAEs [132]. The favourable safety profile that was identified in the Phase II trial was confirmed in this Phase III trial as it was well tolerated [132]. The same conclusions were drawn when analyzing the results in the subgroup of Japanese patients, which was described separately [133]. In this subgroup, zilucoplan also showed a significant improvement in the disease with a favourable safety and tolerability profile [133].

3.3.4. Ongoing Clinical Trials

After approval, clinical trials are still ongoing with zilucoplan to assess the possibility of this drug being used in other situations. A Phase III clinical trial (RAISE-XT) is currently underway, which is an open-label extension study to assess the long-term efficacy of zilucoplan and to continue monitoring its safety and efficacy (NCT04225871) [134]. This trial is expected to be completed in 2026 [135].

There is also an ongoing Phase III trial to assess the safety and tolerability of switching from intravenous C5 inhibitors to the subcutaneous inhibitor (zilucoplan) (NCT05514873) [136].

A Phase II and III trial is underway to study the pharmacokinetics, pharmacodynamics, safety, tolerability, immunogenicity, and activity of zilucoplan in pediatric patients with generalized myasthenia gravis (NCT06055959) [137].

Table 2 summarizes some relevant information regarding all the clinical trials of zilucoplan for generalized myasthenia gravis.

Table 2. Summary of clinical trials of zilucoplan for generalized myasthenia gravis.

Indication	Phase	Status	Location	Identifier
Healthy subjects	I	Completed	-----	-----
Healthy subjects	I	Completed	-----	-----
Generalized myasthenia gravis	II	Completed	USA	NCT03315130
Generalized myasthenia gravis	III	Completed	Multinational	NCT04115293
Generalized myasthenia gravis	III	Active, not recruiting	Multinational	NCT04225871
Generalized myasthenia gravis	III	Active, not recruiting	USA	NCT05514873
Generalized myasthenia gravis	II and III	Not yet recruiting	-----	NCT06055959

4. Zilucoplan and Other Diseases

In addition to the indication for which it was approved, zilucoplan has been the object of clinical trials to assess its effectiveness in other diseases.

A Phase II clinical trial (NCT04025632) was also carried out to assess the efficacy, tolerability, and safety of zilucoplan in patients with immune-mediated necrotising myopathy [138]. It was a multicentre, randomized, double-blind, placebo-controlled study in which 27 patients were enrolled. In this trial, zilucoplan was administered subcutaneously daily for 8 weeks at a dose of 0.3 mg/kg, as this dose had been shown to be effective in inhibiting the terminal complement cascade in patients with generalized myasthenia gravis [138]. As shown in previous trials, zilucoplan was well tolerated by the patients and it was confirmed that zilucoplan can inhibit the terminal complement cascade. Despite this, no improvements were seen in the outcomes assessed for immune-mediated necrotising myopathy, which suggests that activation of the complement cascade is not the main mechanism in this disease [138].

Another Phase II trial (NCT04382755) was carried out with zilucoplan to assess its efficacy and safety in hospitalized COVID-19 patients [139]. It was a multi-center randomized controlled open-label trial in which 32.4 mg of zilucoplan was given subcutaneously every day for 14 days to 81 patients. During this period and for the following 14 days, the patients also received daily 2 g of ceftriaxone intravenously [140].

This study demonstrated that zilucoplan was safe, although it did not show significant improvements in oxygenation parameters after 6 and 15 days of treatment. Further studies will be carried out in larger patient populations to elucidate the clinical efficacy of zilucoplan [140].

Table 3 summarizes some relevant information regarding all the clinical trials of zilucoplan for other diseases.

Table 3. Summary of clinical trials of zilucoplan for other diseases.

Indication	Phase	Status	Location	Identifier
Immune-mediated necrotising myopathy	II	Terminated	Multinational	NCT04025632
COVID-19	II	Completed	Belgium	NCT04382755

5. Conclusions

The treatment of myasthenia gravis is associated with several constraints considering the limitations of the available drugs, as they only treat the symptoms of the disease, are associated to many adverse effects, and can even cause immunogenicity. Therefore, the strategy of using non-antibody inhibitors of the complement system is a good therapeutic solution, as they can directly treat the disease with minor adverse effects. Zilucoplan, a macrocyclic peptide recently approved for treatment of myasthenia gravis, is considered as an orphan drug with an innovative mechanism of action. It is an inhibitor of the terminal complement cascade activation by inhibiting C5. Zilucoplan can be self-administered subcutaneously just once a day and demonstrated efficacy, good tolerability, and safety in several clinical trials. This peptide represents a new hope for the effective treatment of generalized myasthenia gravis, as it can treat the disease with safety and without the risk of immunogenicity. The drug is a welcome addition to targeted biological treatments in MG, being better tolerated and safer than other options, although it was not effective in some 30% of patients in the Phase III RAISE trial; this underlines the fact that other immunological mechanisms beside complement activation are active in myasthenia gravis along with some specific patient-related factors. Furthermore, zilucoplan could also be useful for the treatment of other diseases as well as an inspiration for the synthesis of analogues improving pharmacological profile. Thus, this discovery may open the way for development of other drugs with the same mechanism of action. In addition, this cyclic peptide also evidenced that the chemical class of peptide molecules has a great therapeutic potential to be exploited with one more contribution that reached the therapeutic market with great success.

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