Mechanistic and Pharmacological Differentiation of Zilucoplan, a Macrocyclic Peptide Inhibitor of Complement Component 5 (C5), from Anti-C5 Monoclonal Antibodies

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INTRODUCTION

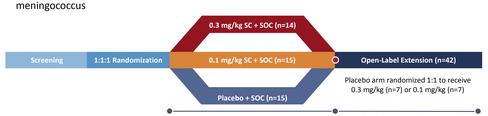
- Zilucoplan is a convenient, subcutaneously self-administered macrocyclic peptide that is designed to bind and inhibit the cleavage of complement component 5 (C5).
- In a Phase 2, randomized, double-blind, placebo-controlled trial for the treatment of acetylcholine receptor (AChR) seropositive generalized myasthenia gravis (gMG), zilucoplan achieved sustained, clinically meaningful, and statistically significant improvements in the key primary and secondary endpoints with a favorable safety and tolerability profile consistent with prior clinical trials, supporting the evaluation of zilucoplan in a Phase 3 trial.
- Zilucoplan is ~40x smaller than eculizumab, a therapeutic anti-C5 monoclonal antibody (mAb), and may exhibit pharmacological and mechanistic differences due to its size and binding properties.

Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of Zilucoplan in a Broad Generalized Myasthenia Gravis Population

Broad Patient Population:
- Generalized myasthenia gravis (MGFA class II-IVa)
- Acetylcholine receptor (AChR) antibody positive
- QMG score of ≥12
- Stable doses of corticosteroids and/or immunosuppressants
- No requirement to be "refractory" or to have failed multiple prior therapies
- Patients must be vaccinated against meningococcus

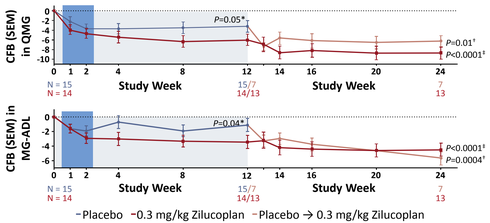
Endpoints:
- Primary: Change in QMG score from baseline to week 12
- Secondary: Change in MG-ADL, MG Composite, and MGQoL15r scores from baseline to week 12
- Prespecified significance testing at a 1-sided alpha of 0.1

Enrollment: 44 Patients (vs target of 36)



MG-ADL, Myasthenia Gravis Activities of Daily Living; MGFA, Myasthenia Gravis Foundation of America; MGQoL15r, 15-item Myasthenia Gravis Quality-of-Life revised scale; QMG, Quantitative Myasthenia Gravis; SC, subcutaneous; SOC, standard of care.

Zilucoplan Achieved Rapid, Clinically Meaningful, Statistically Significant, and Sustained Reductions in QMG and MG-ADL



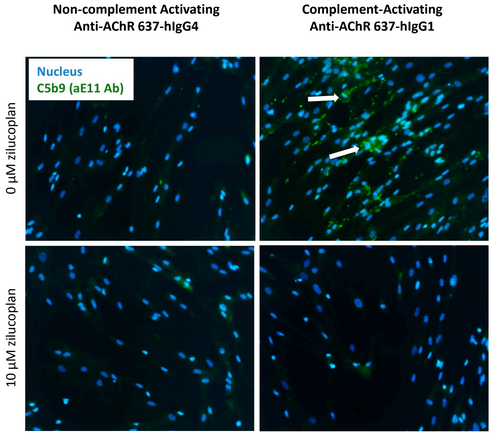
*1-sided analysis of covariance for LS mean change from baseline for the 0.3 mg/kg arm vs placebo; placebo patients re-baselined to zero upon completion of the 12-week main study.
†2-sided *t* test for LS mean change from week 12 to week 24 for placebo patients crossing over to 0.3 mg/kg (n=7).
‡2-sided *t* test for LS mean change from week 0 to week 24 for the 0.3 mg/kg arm.
CFB, change from baseline; LS, least squares; MG-ADL, Myasthenia Gravis Activities of Daily Living; QMG, Quantitative Myasthenia Gravis; SEM, standard error of the mean.
Howard JF Jr, et al. Presented at: American Academy of Neurology Annual Meeting; May 4-10, 2019; Philadelphia, PA.

OBJECTIVES

- Demonstrate mechanism of action of zilucoplan in gMG by assessing the ability of zilucoplan to block insertion of complement components C5b through C9 (C5b-9) induced by anti-AChR antibodies in an in vitro model of the neuromuscular junction (NMJ).
- Investigate pharmacological differentiation between zilucoplan and eculizumab by assessing mechanism of inhibition, including impact of clinically relevant human C5 polymorphisms, in vitro permeability using a basal lamina model, and in vivo clearance of zilucoplan with concomitant anti-neonatal Fc receptor (FcRn) therapy.

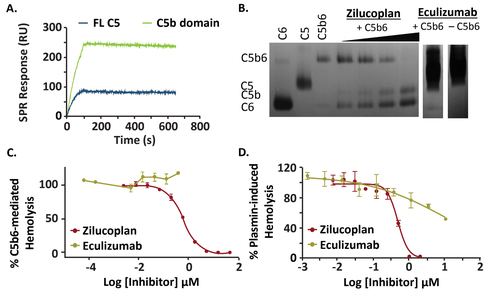
RESULTS

Figure 1. Zilucoplan inhibited C5b-9 deposition induced by anti-AChR antibody in an in vitro NMJ model using co-cultures of human myotubes, neuroblastoma cells, and human sera. Immunofluorescence shows that anti-AChR 637-immunoglobulin (Ig)G1 antibody,[1] but not non-complement activating 637-IgG4, induced C5b-9 deposition on muscle cells that can be blocked with zilucoplan. As complement-mediated destruction of the NMJ contributes to pathogenesis of gMG, these data provide a mechanistic rationale for the clinical response observed in the Phase 2 trial.



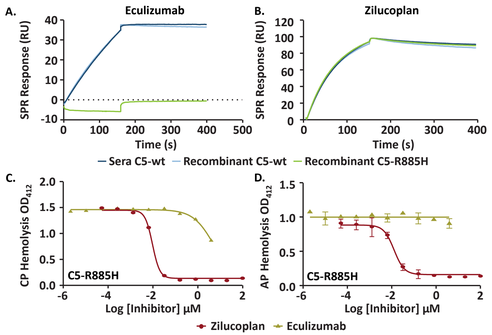
Arrows indicate colocalization of complement-activating anti-AChR antibodies and C5b9 on muscle cells.

Figure 2. Zilucoplan binds a C5b domain and destabilizes C5b6 complex to inhibit non-canonical C5b-9 formation. Zilucoplan binds (A) surface-immobilized full-length human C5 (FL C5) and a C5b domain, (B) dissociates C5b6 complex as demonstrated using native gel analysis, (C) inhibits C5b6-mediated hemolysis, and (D) inhibits plasmin-induced hemolysis. Together these data suggest that zilucoplan, but not eculizumab, can block interaction of C5b with C6 to prevent C5b-9 assembly, including through non-canonical (non-convertase) C5 cleavage.



RU, response unit; SPR, surface plasmon resonance.

Figure 3. Zilucoplan exhibited equipotent binding and inhibition of hemolysis induced by C5 variants reported to be eculizumab resistant. Sensorgrams show that (A) eculizumab does not bind to C5 variant R885H, whereas (B) zilucoplan can bind to both C5 wildtype and R885H variant with equal affinities. Zilucoplan, but not eculizumab, can inhibit hemolysis mediated by C5 variants R885H or R885C (not shown) in classical (C) or alternative (D) pathways. Together, these data suggest that zilucoplan could be efficacious in all patients independent of C5 genotype.



AP, alternative pathway; CP, classical pathway; RU, response unit; SPR, surface plasmon resonance; wt, wildtype.

Figure 4. Greater diffusion of zilucoplan versus eculizumab in an in vitro basal lamina model (Matrigel-coated transwell plates). Pathogenesis of myasthenia gravis involves the localized complement-mediated destruction of the NMJ. The synaptic basal lamina is comprised of extracellular matrix occupying the space in between the nerve terminal and the muscle cell. These data suggest that zilucoplan displays preferential permeability when compared to the mAb eculizumab, and that zilucoplan may more effectively access the NMJ and locally inhibit C5 at the site of action.

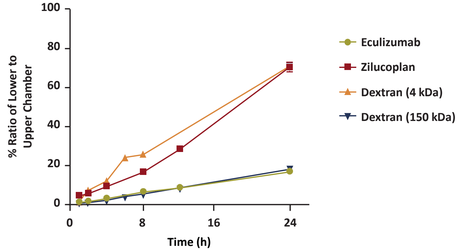


Table 1. Improved biodistribution of low molecular weight zilucoplan in tissues compared with literature values of mAb[2] biodistribution as assessed using quantitative whole-body autoradiography in rats. These data support the possible use of zilucoplan in indications where locally synthesized or activated complement may be involved in the pathogenesis of the disease, including gMG.

	Antibody Biodistribution[2](%)	Zilucoplan Biodistribution (%)
Bone	7.3	15.3
Brain	0.4	0.9
Fat	4.8	16.2
Heart	10.2	22.9
Intestine, large	5.0	21.7
Intestine, small	5.2	10.9
Liver	12.1	27.1
Lung	14.9	37.5
Stomach	5.0	8.5
Lymph nodes	8.5	12.8
Muscle	4.0	7.0
Pancreas	6.4	15.8
Spleen	12.8	15.5
Testes	5.9	15.5
Thymus	6.6	7.8

% of plasma $AUC_{0\text{-}24h}$, area under the curve from 0 to 24 hours.

Figure 5. Anti-FcRn antibody treatment did not impact pharmacokinetics of zilucoplan in non-human primates. Concomitant dosing of zilucoplan and DX-2507[2][3] (A) did not alter anti-FcRn antibody-mediated reduction of IgG or (B) pharmacokinetics of zilucoplan in cynomolgus monkeys. These data suggest that, unlike anti-C5 mAbs, it is possible to use zilucoplan concomitantly with anti-FcRn agents.

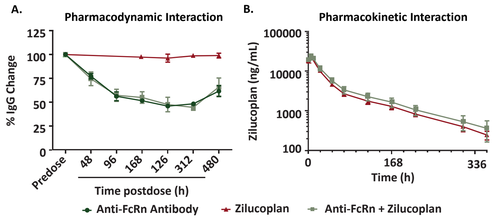
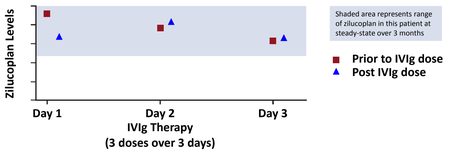


Figure 6. Zilucoplan 0.1 mg/kg levels during IVIg treatment. In the 0.1 mg/kg dose cohort, the dose that was not selected for Phase 3 clinical evaluation, one patient received IVIg therapy while continuing on daily zilucoplan. Levels of zilucoplan did not change during the course of IVIg (measured pre- and post-IVIg). This suggests that unlike anti-C5 mAbs, it is possible to use zilucoplan concomitantly with IVIg agents.



CONCLUSIONS

- Zilucoplan inhibits membrane attack complex deposition on AChR-expressing muscle cells exposed to anti-AChR.
- Unlike eculizumab, zilucoplan is designed to inhibit activation of wildtype, R885C and R885H variants of C5, as well as assembly of C5b-9 complex though destabilizing C5b interaction with C6.
- Zilucoplan showed improved distribution into tissues compared to mAbs and displays superior permeability in a basal lamina model.
- Levels of zilucoplan did not change with concomitant dosing of anti-FcRn antibodies or IVIg, suggesting that unlike anti-C5 mAbs, it is possible to use zilucoplan concomitantly with anti-FcRn or IVIg agents.

REFERENCES

1. van der Neut Kolfschoten M, et al. *Science.* 2007;317:1154-1157.
2. Shah DK, Betts AM. *MAbs.* 2013;5:297-305.
3. Nixon AE, et al. *Front Immunol.* 2015;6:176.

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