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KZR-616, a First-in-class Selective Inhibitor of the Immunoproteasome, Ameliorates Polymyositis in a Murine Model

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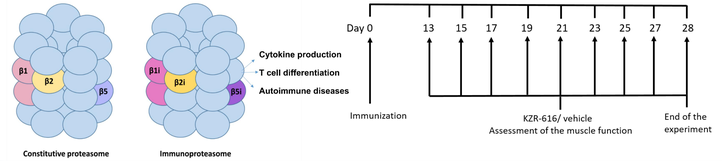
Overview

Polymyositis (PM) is a rare type of idiopathic inflammatory myopathy (IIM). IIMs are a group of muscle diseases that present with muscle weakness and muscle tissue inflammation. Currently, there are no effective targeted therapies for PM except for glucocorticoids and non-specific immunosuppressive drugs. However, response is poor and both treatments contribute to comorbidity. Muscle biopsies suggest an autoimmune pathogenesis as cellular immune infiltrates are detected in multiple foci within the endomysial parenchyma, which consist predominantly of perforin-positive cluster of differentiation (CD)8$^+$ T cells. Experimental PM can be induced in mice by vaccination with fragments of the muscular C protein in a model designated C protein-induced myositis (CIM).[1] As immunoproteasome subunits β1i and β5i are up-regulated at the messenger RNA (mRNA) and protein levels in myofibers and muscle-infiltrating cells in IIMs, immunoproteasome inhibition may have potential for the treatment of inflammatory myositis.[2] Thus, we analyzed the effect of selective immunoproteasome inhibition on the development of PM induced by immunization of mice with a fragment of the myosin binding protein C. Compared with vehicle, treatment with the selective immunoproteasome inhibitors KZR-616 or ONX 0914 (a structural analog of KZR-616) significantly reverted the decreased grip strength associated with the induction of PM and diminished the histopathological score and creatine kinase activity in the serum. This finding suggests that the immunoproteasome may be considered as a potential therapeutic target for the treatment of PM and other autoimmune inflammatory myopathies.

Immunoproteasome inhibition



C-protein–induced myositis

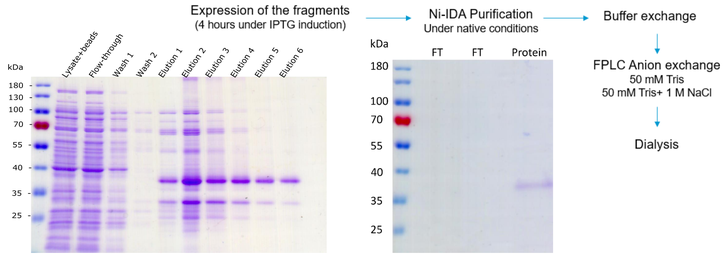


Figure 1. The recombinant skeletal C protein was expressed and purified by a his-tag purification under native conditions. The eluted fractions were further purified by an anion exchange FPLC and dialyzed against 0.5 M arginine, 2 mM reduced glutathione, and 0.2 mM oxidized glutathione in PBS, pH 7.4.
Abbreviations: FPLC, fast protein liquid chromatography; FT, flow-through; IPTG, isopropyl β-D-1-thiogalactopyranoside; Ni-IDA, nickel-iminodiacetic acid; PBS, phosphate-buffered saline.

KZR-616 and ONX 0914 reverted the decreased grip strength and reduced the creatine kinase activity associated with PM

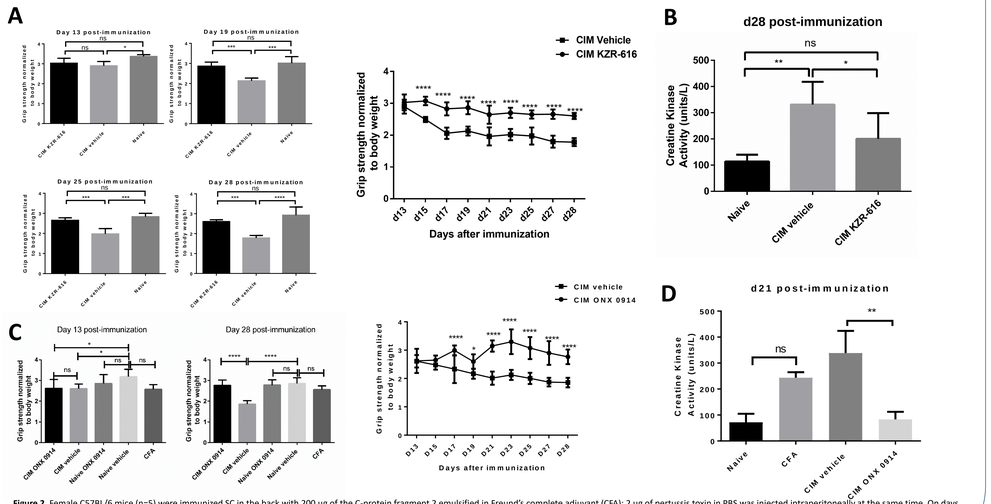
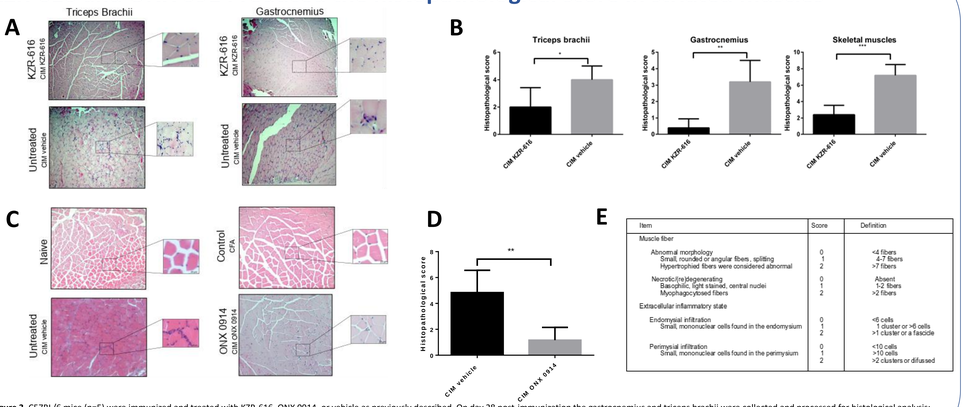


Figure 2. Female C57BL/6 mice (n=5) were immunized SC in the back with 200 µg of the C-protein fragment 2 emulsified in Freund's complete adjuvant (CFA); 2 µg of pertussis toxin in PBS was injected intraperitoneally at the same time. On days 13, 15, 17, 19, 21, 23, 25, and 27 after immunization the mice were treated SC with 10 mg/kg of KZR-616 or vehicle (A) and with 10 mg/kg ONX 0914 or vehicle (C). The muscle strength was assessed with a grip strength meter in a blinded manner and normalized to the body weight. Blood was collected at day 28 post-immunization in the mice treated with KZR-616 or the vehicle (B) and at day 21 post-immunization in the mice treated with ONX 0914 or vehicle. The creatine kinase activity was assessed in serum samples (B and D). *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 from ordinary one-way analysis of variance with a Tukey test.
Abbreviations: CFA, Freund's complete adjuvant; CIM, C-protein–induced myositis; ns, not significant; PBS, phosphate-buffered saline; PM, polymyositis; SC, subcutaneously.

KZR-616 and ONX 0914 reduced the histopathological score in striated muscles



Item	Score	Definition
Muscle fiber		
Abnormal morphology Small, rounded or angular fibers , splitting Hypertrophied fibers were considered abnormal	0 1 2	<4 fibers 4-7 fibers >7 fibers
Necrotic/regenerating Basophilic, light stained, central nuclei Myophagocytosed fibers	0 1 2	Absent 1-2 fibers >2 fibers
Extracellular inflammatory state		
Endomysial infiltration Small, mononuclear cells found in the endomysium	0 1 2	<6 cells 1 cluster or >6 cells >1 cluster or a fascicle
Perimysial infiltration Small, mononuclear cells found in the perimysium	0 1 2	<10 cells >10 cells >2 clusters or diffused

Figure 3. C57BL/6 mice (n=5) were immunized and treated with KZR-616, ONX 0914, or vehicle as previously described. On day 28 post-immunization the gastrocnemius and triceps brachii were collected and processed for histological analysis; 4-µm paraffin embedded sections were collected and stained with hematoxylin–eosin. Representative images of the sectioned muscles are depicted (A, C); the muscles were scored independently (B, D). The mean score of both triceps brachii and gastrocnemius are also shown (B, D). The muscle damage scoring was performed according to the modified criteria from Rizo-Roca et al. (E). *p<0.05, **p<0.01, ***p<0.005 from unpaired t test.
Abbreviations: CFA, Freund's complete adjuvant; CIM, C-protein–induced myositis.

LMP7 is required for CIM induction

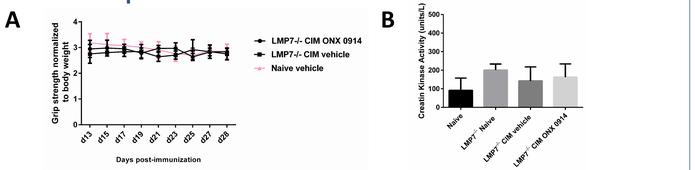
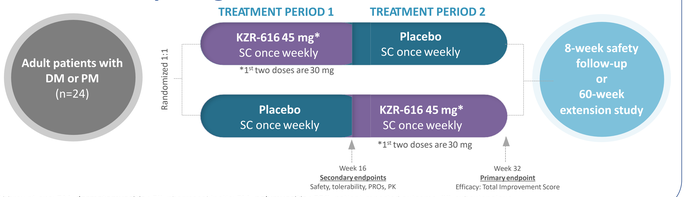


Figure 4. Female LMP7$^{-/-}$ mice (n=4) were immunized SC in the back with 200 µg of the C-protein fragment 2 emulsified in Freund's complete adjuvant. 2 µg of pertussis toxin in PBS was injected intraperitoneally at the same time. On days 13, 15, 17, 19, 21, 23, 25, and 27 after immunization, the mice were treated SC with 10 mg/kg ONX 0914 or vehicle. The muscle strength was assessed with a grip strength meter in a blinded way and normalized to the body weight (A). The creatine kinase activity was assessed in serum samples (B). An ordinary two-way analysis of variance with a Tukey test was performed.
Abbreviations: CFA, Freund's complete adjuvant; CIM, C-protein–induced myositis; LMP7, low-molecular mass protein 7; PBS, phosphate-buffered saline; SC, subcutaneously.

Summary and outlook

- Two-step protein purification achieves high yield and purity of the MYBPC2 fragment
- Immunoproteasome inhibition reverted the decreased grip strength associated with PM and the creatine kinase activity in the serum
- KZR-616 or ONX 0914 treatment reduced the histopathological score of the inflamed striated muscles
- Deficiency of immunoproteasome subunit LMP7 impairs the induction of CIM
- These results provide a rationale for the currently enrolling PRESIDIO study (NCT: NCT04033926), a phase 2 placebo-controlled, crossover study of KZR-616 for patients with PM and dermatomyositis (DM) that is evaluating safety, tolerability, and exploratory efficacy outcomes such as muscle function and disease activity over 32 weeks of treatment

PRESIDIO study design



Abbreviations: DM, dermatomyositis ; PK, pharmacokinetic; PM, polymyositis ; PRO, patient-reported outcome; SC, subcutaneious.

References

1. Sugihara T, et al. *Arthritis Rheum*. 2007;56(4):1304-1314.
2. Bhattarai S, et al. *J Autoimmun*. 2016;75:118-129.
3. Rizo-Roca D, et al. *Eur J Histochem*. 2015;59(4):2544.

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