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Structure-Function Analysis of ARGX-119, a First-in-Class Humanized Agonist Monoclonal Antibody Specific for Muscle-Specific Kinase

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BACKGROUND

Muscle-specific kinase

- MuSK plays a crucial role in the formation and maintenance of neuromuscular synapses, and is essential for agrin-induced AChR clustering during NMJ formation[1–3]
- The MuSK Fz-like domain (**Figure 1**) is dispensable for synapse formation and maturation[2,3]
- The crystal structure of the Fz-like domain of MuSK was previously determined in rats[4]
- Agonist antibodies that bind the MuSK Fz-like domain have shown therapeutic potential in preclinical models[5,6]
  - However, these antibodies either recognize proteins in addition to MuSK or fail to recognize human MuSK[3]

FIGURE 1 MuSK Domain

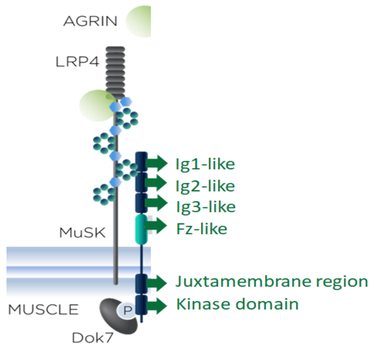
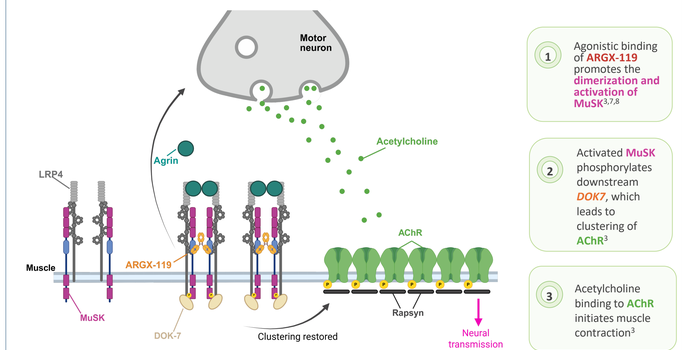


FIGURE 2 ARGX-119 Proposed Mechanism of Action



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ARGX-119

- ARGX-119 is the first fully humanized monoclonal antibody that binds with high affinity to the Fz-like domain of human, non-human primate, rat, and mouse MuSK, without off-target binding, making it suitable for clinical development **(Figure 2)**[3]

Objective

- To elucidate the crystal structure of the ARGX-119 Fab fragment in complex with the human MuSK Fz-like domain

METHODS

Crystallization

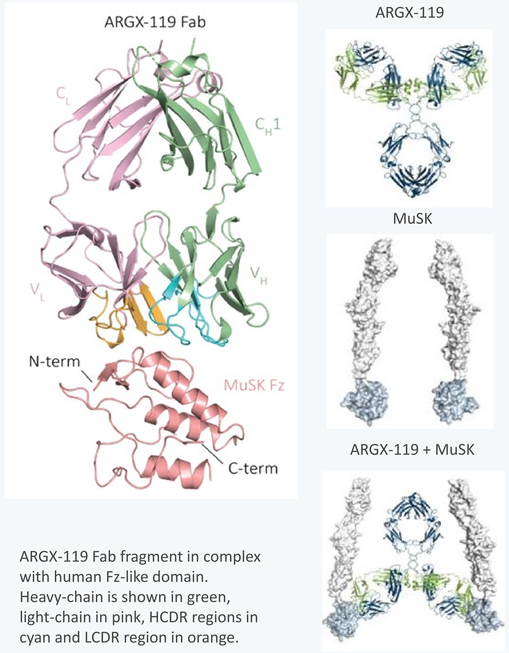
- Crystals were prepared according to established protocols
  - Diffraction data were collected at ESRF (Grenoble, France)
  - Crystals diffracted anisotropically to a resolution of 2.29 Å

Binding analyses

- Constructs of the human Fz-like domain were designed; corresponding proteins produced in HEK293 cells were tested for binding against the Fab of ARGX-119 on SPR and binding ELISA to identify the minimal construct

RESULTS

FIGURE 3 Crystal Structure of ARGX-119:MuSK Fz-Like Domain



ARGX-119 Fab fragment in complex with human Fz-like domain. Heavy-chain is shown in green, light-chain in pink, HCDR regions in cyan and LCDR region in orange.

Crystallization

- For the first time, a human Fz-like domain was crystallised, and the MuSK Fz-like domain was co-crystallised with ARGX-119 IgG Fab fragment (**Figure 3**)

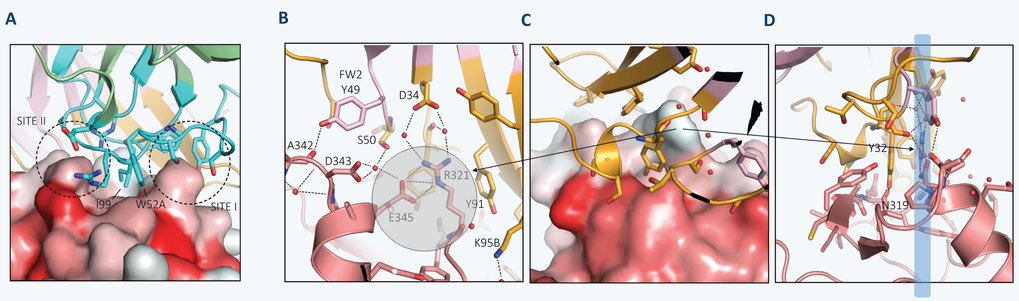
ARGX-119:MuSK Fz-like interaction

- Both the heavy and light chain collectively contribute to the binding of the Fz-like domain (**Figure 4**)

A unique binding interface due to electrostatic network interaction

- In-depth analysis revealed that the light chain of ARGX-119 engages with the MuSK Fz-like domain via a remarkably dense network of electrostatic interactions
- The VH-epitope features 2 sites of electrostatic interactions, separated by a hydrophobic ridge, to bind the Fz-like region (**Figure 4A**)
- The electrostatic network provides critical support for VL to expand an electrostatic wall of hydrogen bonds (dashed lines), and for LCDR1 Y32 to dive into a hydrophobic groove of the MuSK Fz-like domain, and anchor at the hydroxyl tip via a hydrogen bond with the main chain of Fz N319 (**Figures 4B–D**)
- The importance of this electrostatic network was demonstrated by the observation that a single substitution (E345K), detected in the CD-1 mouse strain, influenced the binding of ARGX-119 to MuSK (**Figure 4B**)
  - Q345K has an electrostatic (lysine is positively charged) and size mismatch that disrupts key hydrogen bonds, affecting conformation and therefore affinity to MuSK

FIGURE 4 Analysis of VH (A) and VL (B–D) Epitope Binding



Heavy-chain is shown in green, light-chain in pink, HCDR regions in cyan and LCDR region in orange.

KEY TAKEAWAYS

The ARGX-119 VH-epitope features two sites of electrostatic interactions, separated by a hydrophobic ridge, to bind the Fz-like region

Together, the ARGX-119 VH and VL engage the N-terminal 'tip' of the Fz-like domain in a continuous epitope

The crystal structure explains why ARGX-119 exhibits reduced binding affinity to CD-1 mouse MuSK due to the E345K mutation in the Fz-like domain

These results provide a better understanding of the MoA of ARGX-119 and highlight the relevance of sequencing the target protein in the selected species during preclinical evaluations

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ABBREVIATIONS

AChR, acetylcholine receptor; C_H, constant domain of heavy chain; C_L, constant domain of light chain; *DOK7*, docking protein 7; ELISA, enzyme-linked immunosorbent assay; ESRF, European Synchrotron Radiation Facility; Fab, fragment antigen-binding; Fz-like, Frizzled-like; Ig1/2/3-like, immunoglobulin-like domains 1/2/3; HCDR, heavy-chain complementarity-determining region; IgG, immunoglobulin G; LCDR, light-chain complementarity-determining region; LRP4, low-density lipoprotein receptor-related protein 4; MoA, mechanism of action; MuSK, muscle-specific kinase; NMJ, neuromuscular junction; SPR, surface plasmon resonance; V_H, variable domain of heavy chain; V_L, variable domain of light chain.

DISCLOSURES AND ACKNOWLEDGMENTS

CS, EP, BV, MP, LDC, RC, KM and RV: Employees: argenx; **MGH:** Employee: Leiden University Medical Center, an inventor on MuSK-related patents (both MGH and Leiden University Medical Center receive royalties from these patents, with Leiden University Medical Center receiving royalties over a MuSK ELISA); Consultant: argenx; **SJB:** Employee: MGH/Harvard University; Holds the following patents: US9329182, US20150125442A1, and US11492401.

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