MuSK-CAART: A novel precision cellular therapy for muscle-specific tyrosine kinase myasthenia gravis

Sangwook Oh, PhD; Aimee S. Payne, MD, PhD

Department of Dermatology, Center for Cellular Immunotherapies, University of Pennsylvania

ABSTRACT

Chimeric autoantibody receptor (CAAR) T cells are a novel genetically-engineered T cell immunotherapy that aims to durably eliminate antigen-specific B cells while sparing healthy B cells, ideally leading to safe and lasting remission of B cell-mediated autoimmune diseases with a one-time infusion. We describe the preclinical development of muscle-specific tyrosine kinase CAAR T cells (MuSK-CAART) for the treatment of MuSK myasthenia gravis, a debilitating autoantibody-mediated disease that causes potentially life-threatening muscle weakness.

Key words: autoimmunity, neurology, immunotherapy, CAR T cells, neuromuscular junction, MuSK, myasthenia gravis, muscle-specific tyrosine kinase.

Chimeric antigen receptor (CAR) T cells are genetically-engineered cellular immunotherapies that have led to durable remissions of otherwise refractory B cell malignancies. Four CD19-targeted CAR T cell products are clinically approved for the treatment of B cell leukemias and lymphomas, based on pivotal trials in which 53-81% of clinical trial participants achieved complete remission and 40-57% demonstrated long-term remission (1-4), including several that are thought to have achieved cancer cure. However, CAR T cell therapy can cause serious and potentially fatal side effects, including cytokine release syndrome, resulting from the rapid immune activation and tumor cell death that occurs after infusion, immune effector cell-associated neurotoxicity syndrome, and infections from B cell depletion.

Nevertheless, the remarkable success of CAR T cell therapy in B cell-mediated cancers inspired us to consider other B cell-mediated diseases that could be treated with a similar therapeutic approach. Muscle-specific tyrosine kinase (MuSK) myasthenia gravis is a B cell-mediated autoimmune disease in which autoantibodies against the postsynaptic transmembrane protein MuSK interfere with neuromuscular junction signaling, resulting in muscle weakness. Patients with MuSK myasthenia gravis can have difficulty swallowing, speaking, moving, or breathing, which can advance to life-threatening respiratory crisis. Currently, there are no FDA-approved treatments specific for MuSK myasthenia gravis, although corticosteroids and rituximab are considered front-line therapies (5). Anti-MuSK antibody titers drop after rituximab therapy (6), indicating that short-lived plasma cells produce anti-MuSK antibodies (7, 8) and that strategies to deplete anti-MuSK memory B cell precursors should prevent anti-MuSK antibody production.

We therefore re-engineered CAR T cells for antigen-specific B cell depletion in MuSK myasthenia gravis. Anti-CD19 CAR T cells incorporate an anti-CD19 antibody as the extracellular domain of the CAR, linked to cytoplasmic co-stimulatory and activation domains. This approach targets CD19-expressing B cells, both healthy and leukemic, and can lead to B cell cancer remission and potentially lifelong B cell depletion due to the induction of memory CAR T cells (**Figure 1A**). To target only the anti-MuSK B cells in MuSK myasthenia gravis, we expressed the MuSK autoantigen ectodomain on the surface of T cells, linked to CD137 costimulatory and CD3ζ activation domains (**Figure 1B**). This chimeric autoantibody receptor (CAAR) is designed to target the anti-MuSK B cell receptor, which is identical in specificity to the autoantibody the B cell will produce once activated to mature into an antibody-secreting cell. Ideally, MuSK CAAR T cells (MuSK-CAART) will kill all anti-MuSK B cells to achieve complete remission of MuSK myasthenia gravis and also produce memory CAAR T cells to provide potentially lifelong protection against autoimmune disease recurrence.

The MuSK extracellular domain is comprised of three immunoglobulin (Ig)-like and one frizzled (Fz)-like domain, the entirety of which was incorporated into the MuSK CAAR ectodomain. Using in vitro killing assays against B cells engineered to express anti-MuSK B cell receptors targeting all 3 Ig-like and Fz-like MuSK domains, we demonstrated that MuSK-CAART specifically lyses anti-MuSK B cells. We evaluated MuSK-CAART in vivo efficacy in a syngeneic experimental autoimmune myasthenia gravis model induced by immunization of C57BL/6J mice with the human MuSK ectodomain, followed 5 weeks later with non-transduced T cells, anti-CD19 CART, or MuSK-CAART treatment (**Figure 2A**). Anti-CD19 CART treatment fully depleted splenic B cells, whereas MuSK-CAART did not affect splenic B cells relative to non-transduced T cell treatment (**Figure 2B**) since anti-MuSK B cells are rare in these immunized mice (less than 2% of total splenic B cells). Accordingly, anti-CD19 CART reduced both total serum IgG and anti-MuSK IgG, whereas MuSK-CAART reduced anti-MuSK IgG without effect on total serum IgG levels

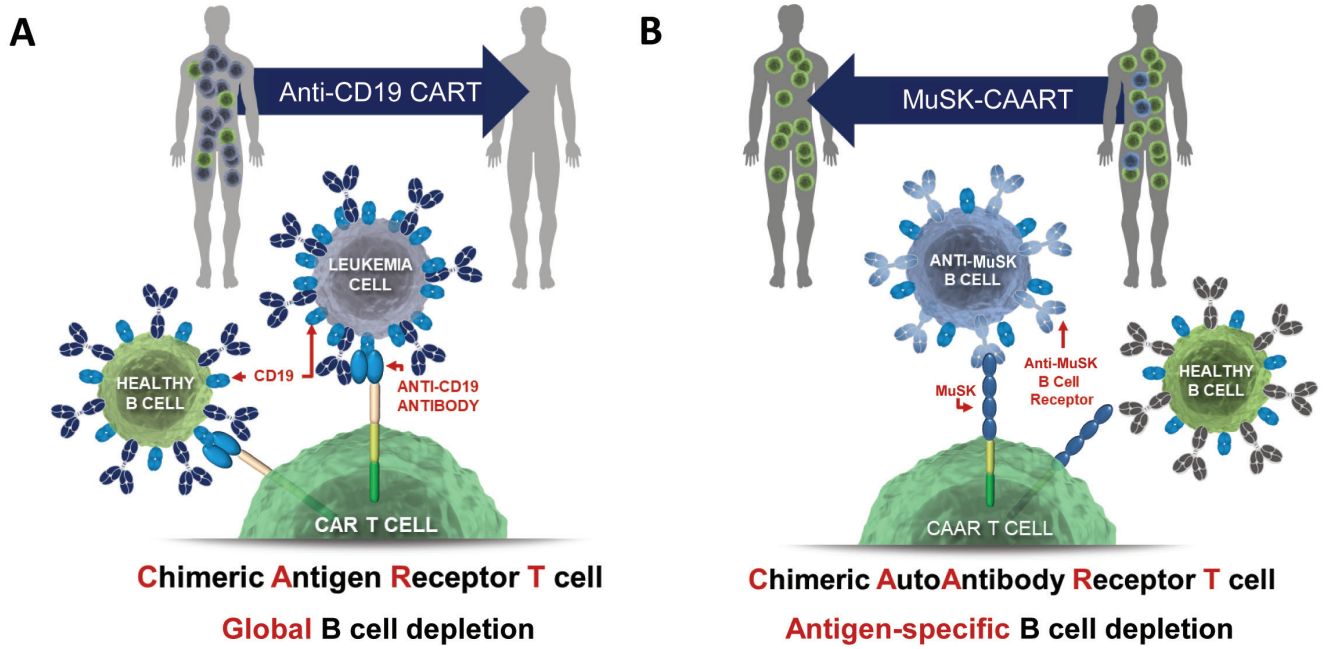

Figure 1. Schematic of chimeric antigen receptor (CAR) T cells versus chimeric autoantibody receptor (CAAR) T cells. A) CAR T cells clinically approved to treat B cell cancers incorporate an anti-CD19 antibody as the extracellular domain of a chimeric immunoreceptor, linked to cytoplasmic co-stimulatory and activation domains. Anti-CD19 CAR T cells kill both healthy and leukemic CD19-expressing B cells, leading to complete and durable cancer remission through global B cell depletion. B) Muscle-specific tyrosine kinase (MuSK) chimeric autoantibody receptor (CAAR) T cells incorporate the MuSK autoantigen targeted in MuSK myasthenia gravis, tethered to cytoplasmic co-stimulatory and activation domains. MuSK CAAR T cells are designed to specifically deplete anti-MuSK B cells that express an anti-MuSK B cell receptor, while sparing healthy B cells, which ideally will lead to durable remission of MuSK myasthenia gravis without global immune suppression. Image credit: Adapted with permission from Cabaletta Bio.

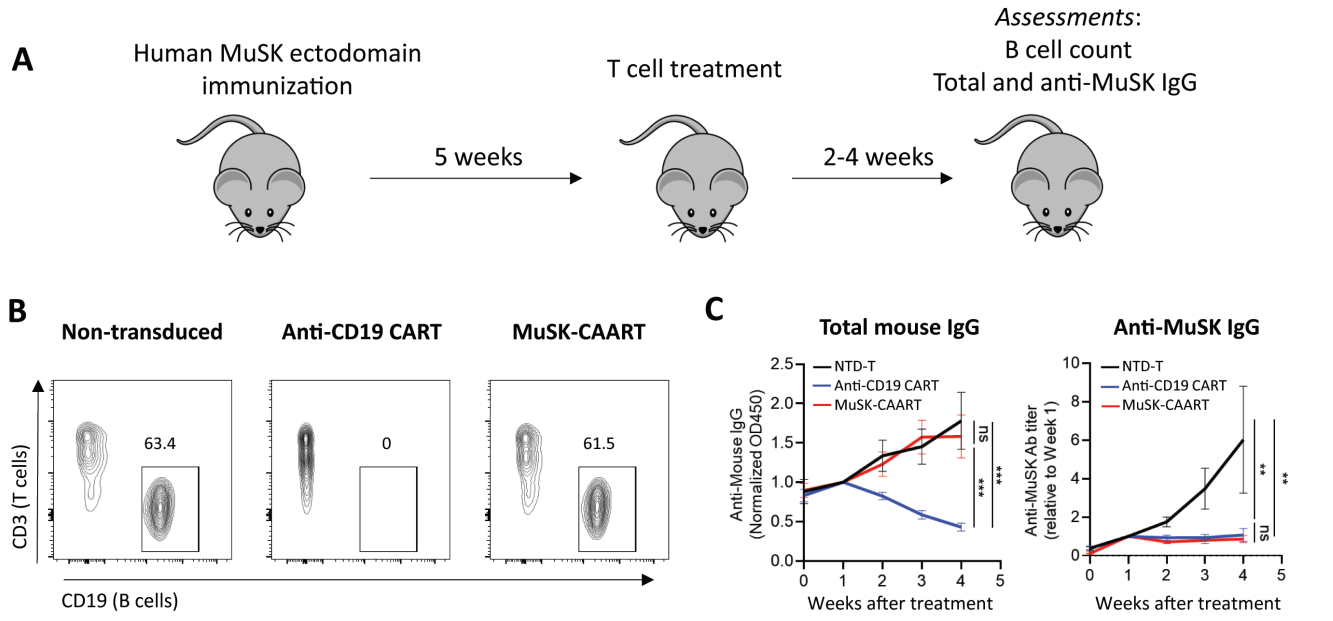

Figure 2. MuSK CAAR T cell therapy of experimental MuSK myasthenia gravis leads to antigen-specific B cell depletion. A) Experimental design: Mice are immunized with human MuSK ectodomain to induce anti-MuSK IgG, followed 5 weeks later by MuSK-CAART treatment. Mice were analyzed 2 weeks later by flow cytometry of spleen and 4 weeks later for total serum IgG or anti-MuSK IgG. B) CD19-expressing B cells are depleted by anti-CD19 CAR T cell treatment (middle panel) but not MuSK CAAR T cell (right panel) or non-transduced T cell treatment (left panel). C) Anti-CD19 CAR T cells (shown in blue) deplete total and MuSK-specific IgG, whereas MuSK CAAR T cells deplete only anti-MuSK IgG (shown in red), while preserving total IgG levels. Non-transduced T cells (NTD-T shown in black) do not deplete total or MuSK-specific IgG.

(**Figure 2C**), indicating antigen-specific B cell depletion.

We examined the potential for unwanted off-target effects of MuSK-CAART through several approaches, including comprehensive organ histology and serum chemistry in MuSK-CAART treated mice (an approach expected to yield information on potential off-target interactions mediated by the MuSK ectodomain given the high homology between mouse and human MuSK), high-throughput screening of membrane proteome arrays expressing greater than 5,300 human membrane proteins, and screening of human primary cell cultures and primary human myotubes for evidence of MuSK-CAART activation after co-incubation. Specific off-target cytotoxic interactions of MuSK-CAART were not identified in these assays.

The complete description of the MuSK-CAART design, as well as evaluations of its efficacy and safety were recently published (9). Collectively, these studies contributed to an Investigational New Drug application for MuSK-CAART and have led to an open label phase 1 study to evaluate the safety and preliminary efficacy of various dosing regimens of MuSK-CAART for MuSK myasthenia gravis (NCT05451212), which is currently recruiting. Participants must be age 18 or older, have active disease (class I-IVa as assessed by the MGFA (Myasthenia Gravis Foundation of America) Clinical Classification), and have a positive anti-MuSK antibody titer. Participants must not have received rituximab in the past 12 months, be on a prednisone dose greater than 0.25 mg/kg/day, or have another disease requiring immunosuppressive therapy. Immunosuppressives used for MuSK myasthenia gravis will be stopped or tapered prior to MuSK-CAART infusion. The primary endpoint of the study will be related adverse events, including dose-limiting toxicities, up to 3 months after MuSK-CAART infusion. Secondary outcomes include MuSK-CAART persistence and change in MuSK autoantibody titer compared to pre-infusion. Exploratory outcomes include frequency and dose of concomitant therapies, clinical disease activity and quality of life measurements.

In summary, MuSK-CAART represents a novel precision cellular immunotherapy for MuSK myasthenia gravis. Ongoing clinical studies will evaluate its potential for safe and durable autoimmune disease remission.

Corresponding Author
Aimee S. Payne, MD, PhD
1009 Biomedical Research Building
421 Curie Boulevard
Philadelphia, PA 19104
Telephone: 215-746-4488
Email: aimee.payne@pennmedicine.upenn.edu

References

1. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-48. doi: 10.1056/NEJMoa1709866.

2. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. Lancet Oncol. 2019;20(1):31-42. doi: 10.1016/S1470-2045(18)30864-7.

3. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. N Engl J Med. 2020;382(14):1331-42. doi: 10.1056/NEJMoa1914347.

4. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020;396(10254):839-52. doi: 10.1016/S0140-6736(20)31366-0.

5. Narayanaswami P, Sanders DB, Wolfe G, Benatar M, Cea G, Evoli A, et al. International Consensus Guidance for Management of Myasthenia Gravis: 2020 Update. Neurology. 2021;96(3):114-22. doi: 10.1212/WNL.0000000000011124.

6. Marino M, Basile U, Spagni G, Napodano C, Iorio R, Gulli F, et al. Long-Lasting Rituximab-Induced Reduction of Specific-But Not Total-IgG4 in MuSK-Positive Myasthenia Gravis. Front Immunol. 2020;11:613. doi: 10.3389/fimmu.2020.00613.

7. Stathopoulos P, Kumar A, Nowak RJ, O'Connor KC. Autoantibody-producing plasmablasts after B cell depletion identified in muscle-specific kinase myasthenia gravis. JCI Insight. 2017;2(17). doi: 10.1172/jci.insight.94263.

8. Zografou C, Vakrakou AG, Stathopoulos P. Short- and Long-Lived Autoantibody-Secreting Cells in Autoimmune Neurological Disorders. Front Immunol. 2021;12:686466. doi: 10.3389/fimmu.2021.686466.

9. Oh S, Mao X, Manfredo-Vieira S, Lee J, Patel D, Choi EJ, et al. Precision targeting of autoantigen-specific B-cells in muscle-specific tyrosine kinase myasthenia gravis with chimeric autoantibody receptor T-cells. Nat Biotechnol. 2023; doi: 10.1038/s41587-022-01637-z.

Acknowledgements

We are grateful to our colleagues and collaborators Xuming Mao, Silvio Manfredo-Vieira, Jinmin Lee, Darshil Patel, Eun Jung Choi, Andrea Alvarado, Ebony Cottman-

Thomas, Damian Maseda, Patricia Y. Tsao, Christoph T. Ellebrecht, Sami L. Khella, David P. Richman, Kevin C. O'Connor, Uri Herzberg, Gwendolyn K. Binder, Michael C. Milone, and Samik Basu. The research described was supported by a sponsored research award from Cabaletta Bio (A.S.P.), the National Institute of Allergy and Infectious Diseases of the National Institutes of Health through grant awards to K.C.O. (R01-AI114780 and R21-AI142198) and a sponsored research subaward from the University of Pennsylvania, the primary financial sponsor of which is Cabaletta Bio. S.O. was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by the Ministry of Education (2019R1A6A3A03033057).

Conflict of Interest

SO: Cabaletta Bio: Patent licensing

ASP: Cabaletta Bio: equity, payments, research support, patent licensing; Janssen: consultant