

MuSK chimeric autoantibody receptor (CAAR) T cells for antigen-specific cellular immunotherapy of myasthenia gravis

Sangwook Oh¹, Kevin C. O'Connor², and Aimee S. Payne¹

¹Department of Dermatology, University of Pennsylvania, Philadelphia, PA

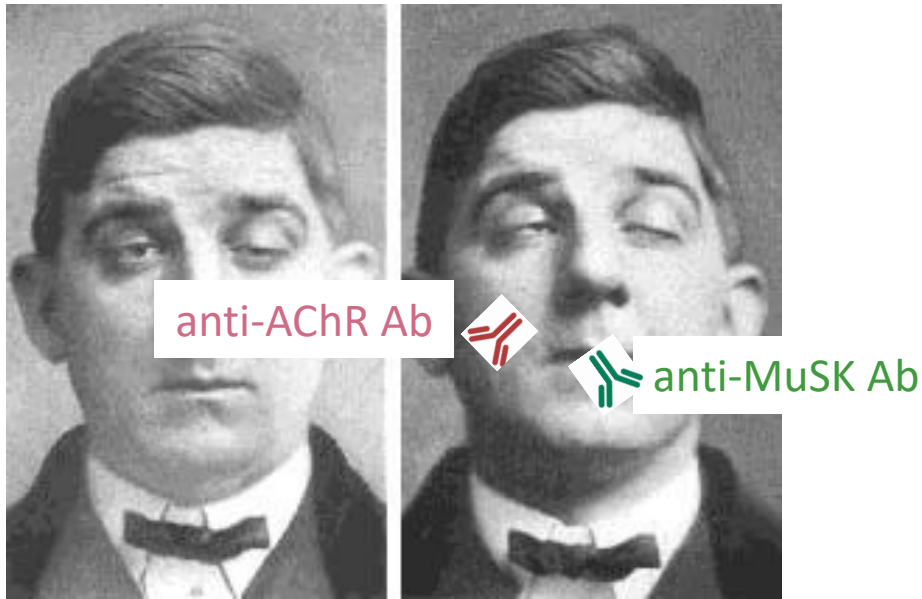
²Department of Neurology and Immunobiology, Yale University, New Haven, CT

Conflicts of interest:

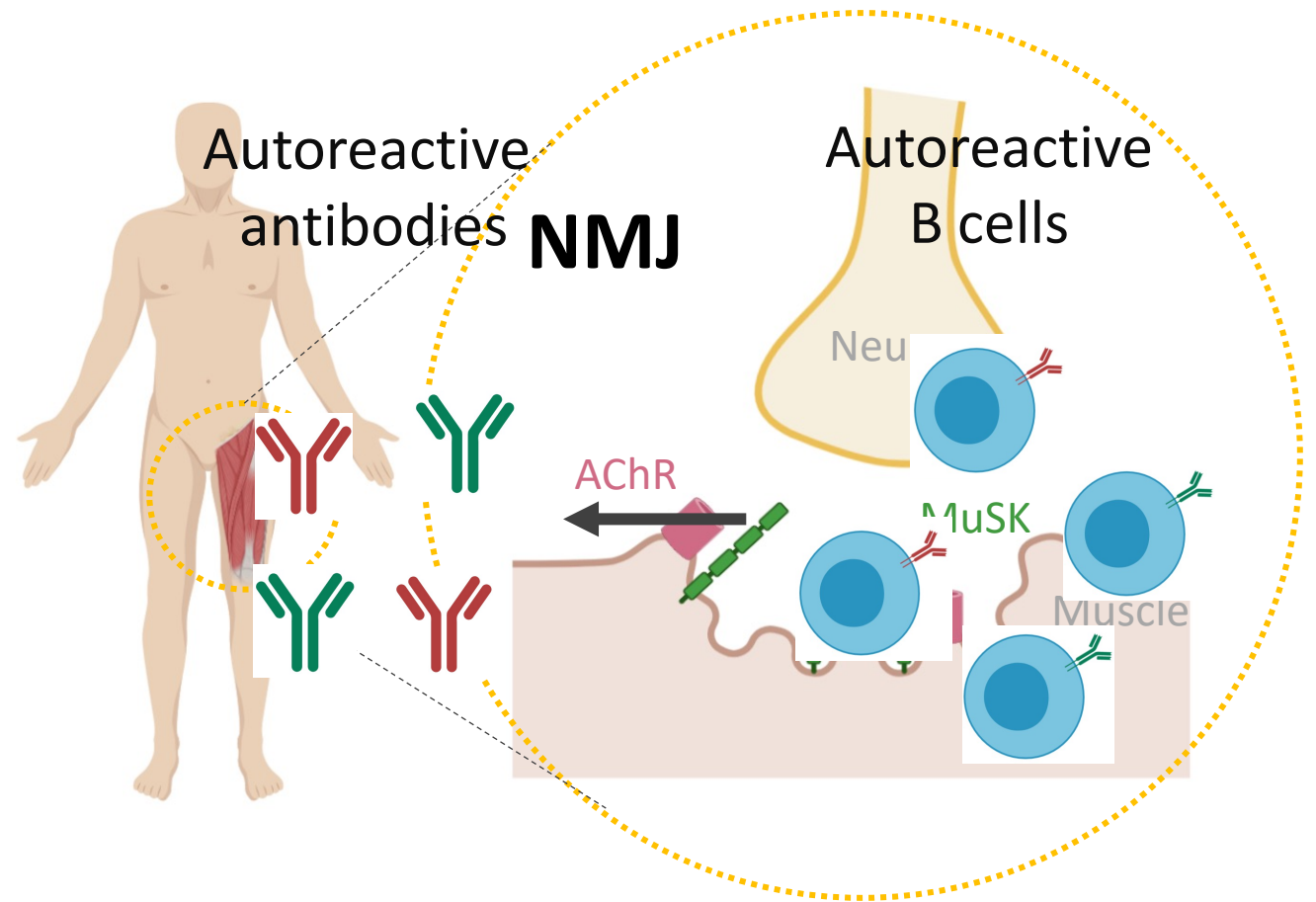
- Oh: inventor on patents licensed by Cabaletta Bio
- O'Connor: consultant, Cabaletta Bio, Ra Pharma
- Payne: co-founder, co-chair SAB, Cabaletta Bio; inventor on patents licensed by Cabaletta Bio, Novartis



Myasthenia gravis (MG) is a B cell-mediated autoimmune disease of the neuromuscular junction (NMJ)



Ptosis in a patient with MG
(Posey & Spiller, ed. 1904)

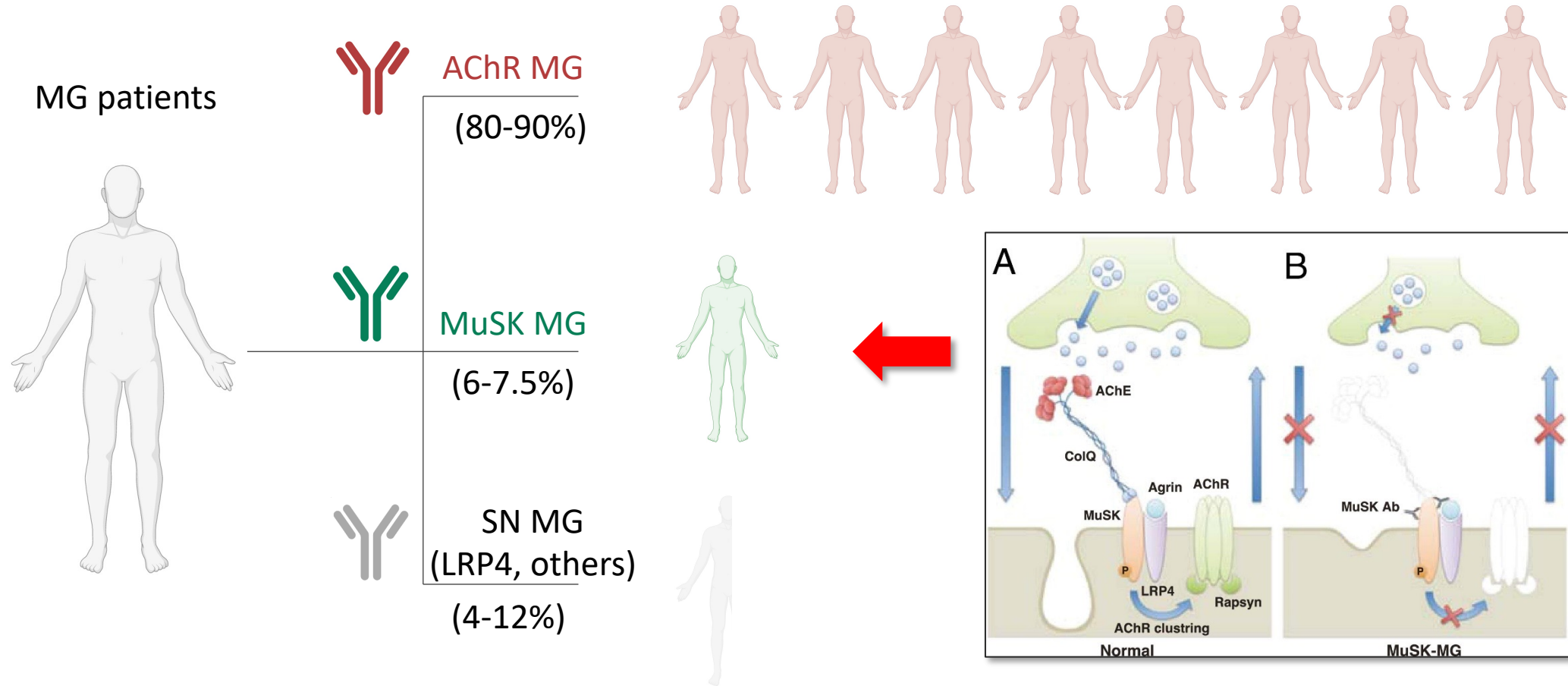


Autoantibodies produced by autoreactive B cells block AChR and MuSK at the NMJ



MG has at least three subtypes based on autoantibody profile

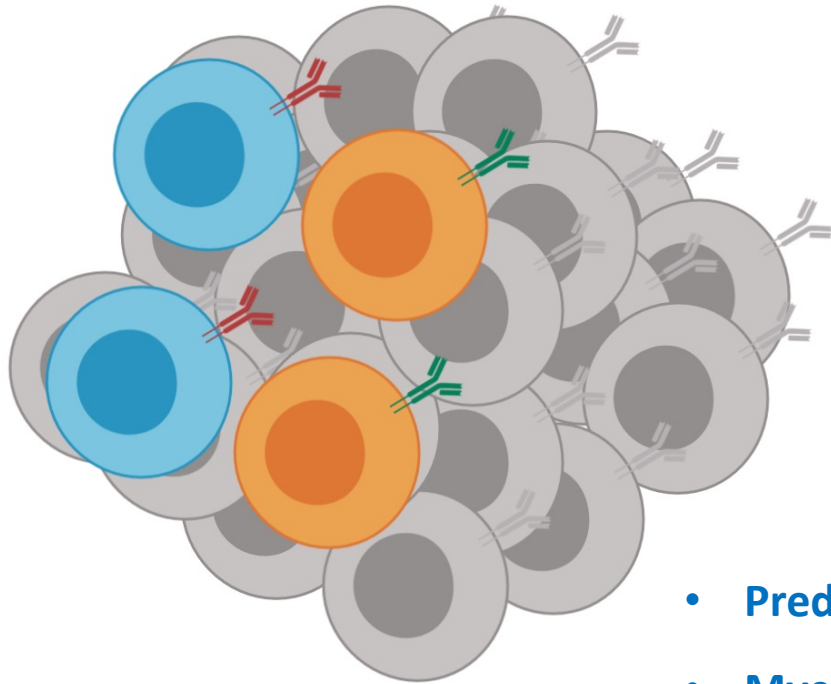
MuSK is a key protein for **AChR clustering** which is critical for neuronal signal transduction



* Seronegative (SN): anti-AChR & anti-MuSK Ab test negative

Most treatments for MuSK MG involve off-label immunosuppressants

Autoreactive B cells



However,

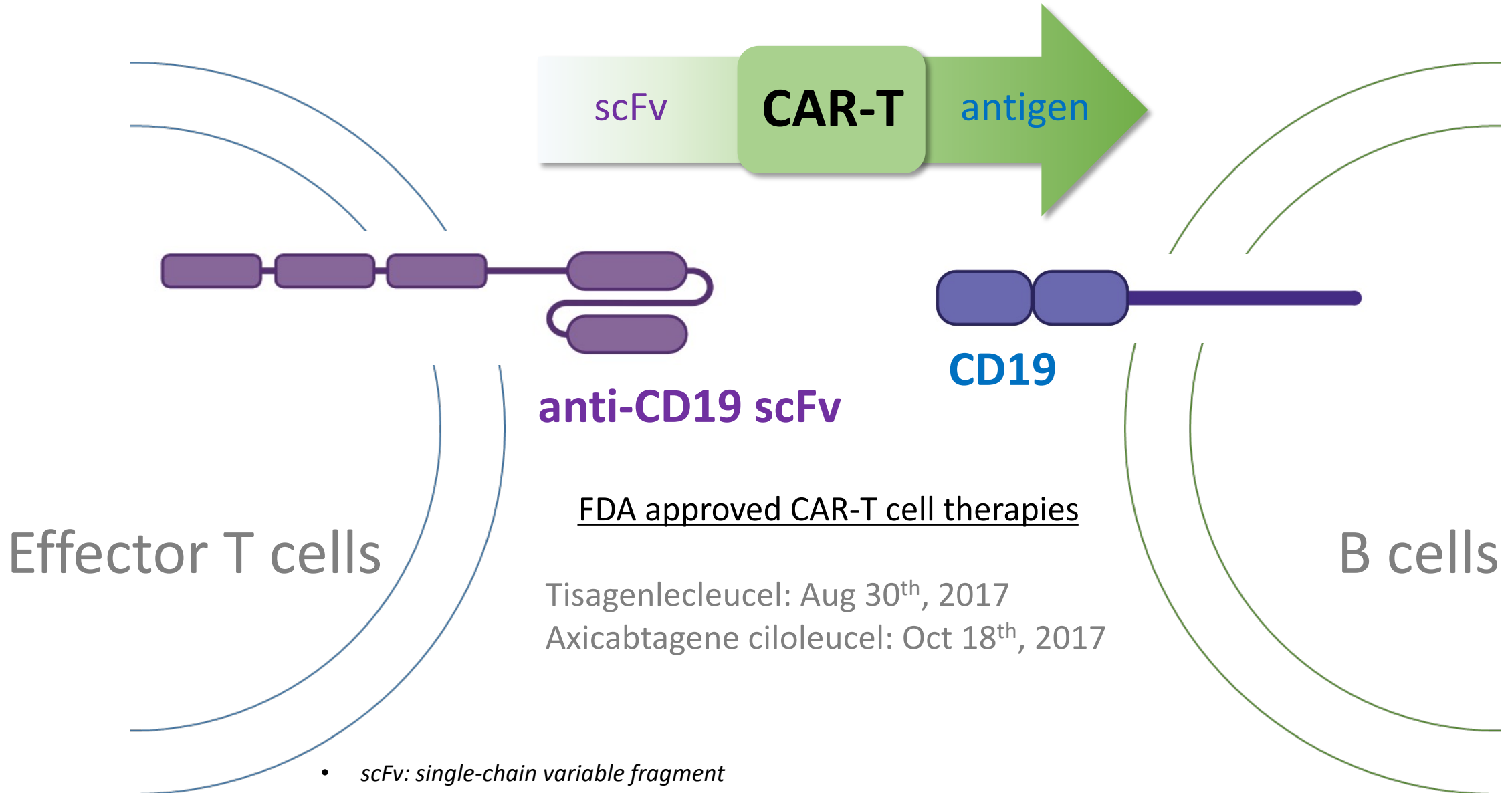
Immunosuppressants target normal B cells, which increases *risk of infection*

Chronic immunosuppression or repeated infusions are often necessary to maintain disease control

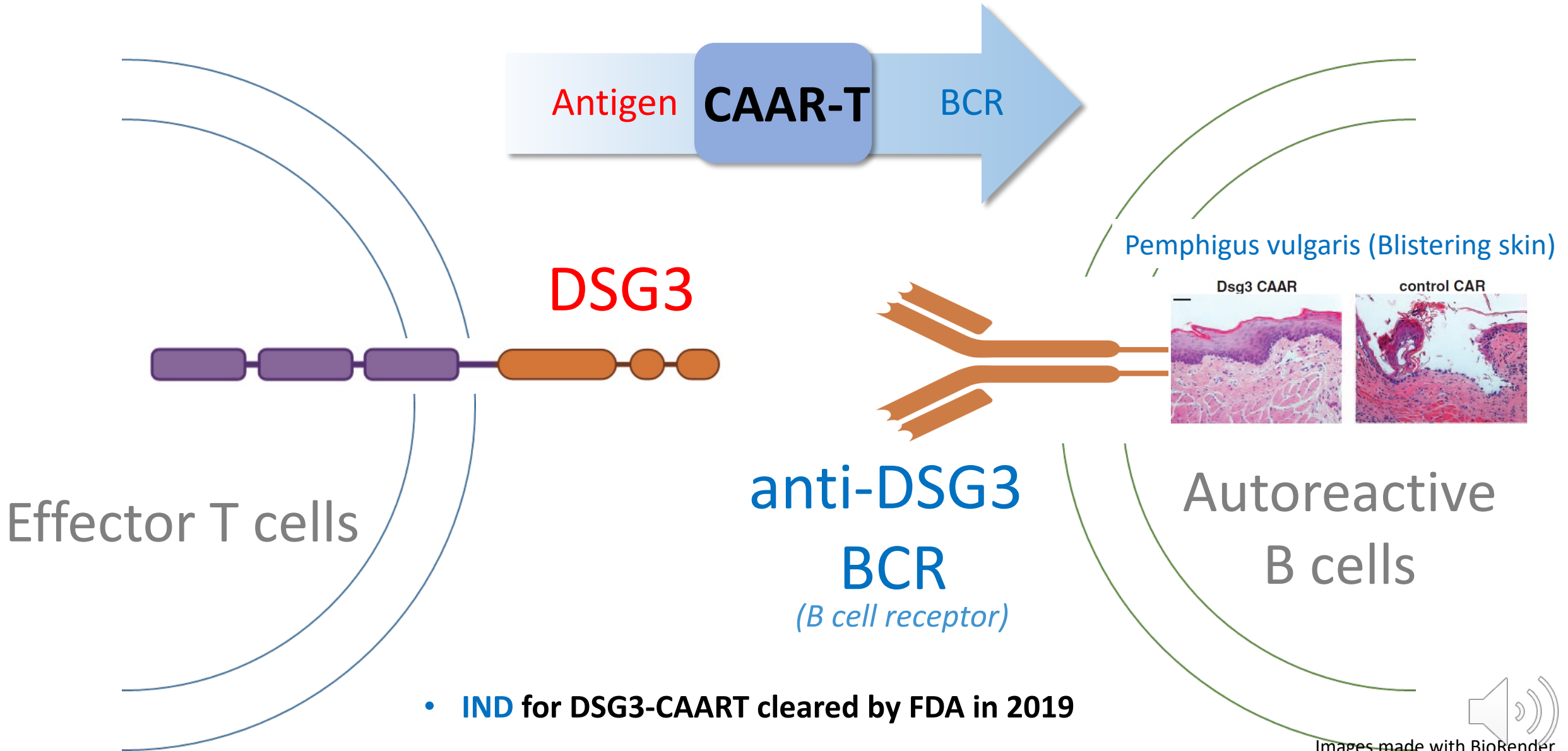
- **Prednisone:** global immunosuppressant
- **Mycophenolate mofetil/azathioprine:** preferentially inhibits lymphocytes
- **Rituximab:** anti-CD20 monoclonal antibody targeting B cells



Success of Chimeric Antigen Receptor (CAR) T cell therapy for B cell leukemias and lymphomas



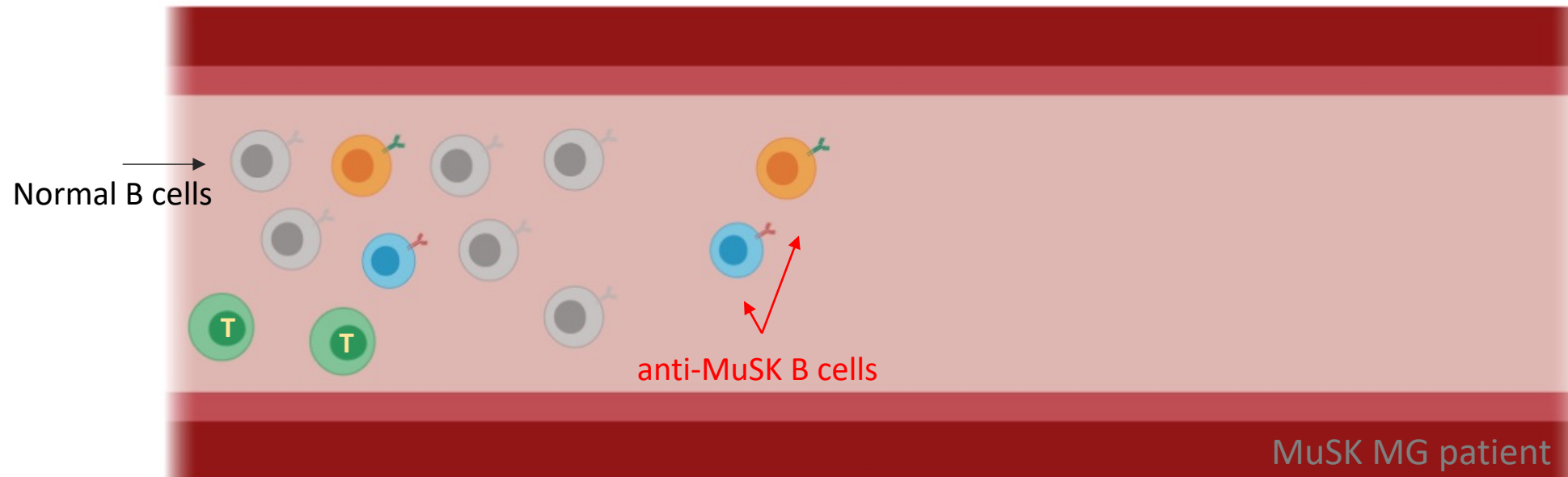
Chimeric autoantibody receptor (CAAR) T cells are designed to specifically target antigen specific B cells



- **IND** for DSG3-CAART cleared by FDA in 2019

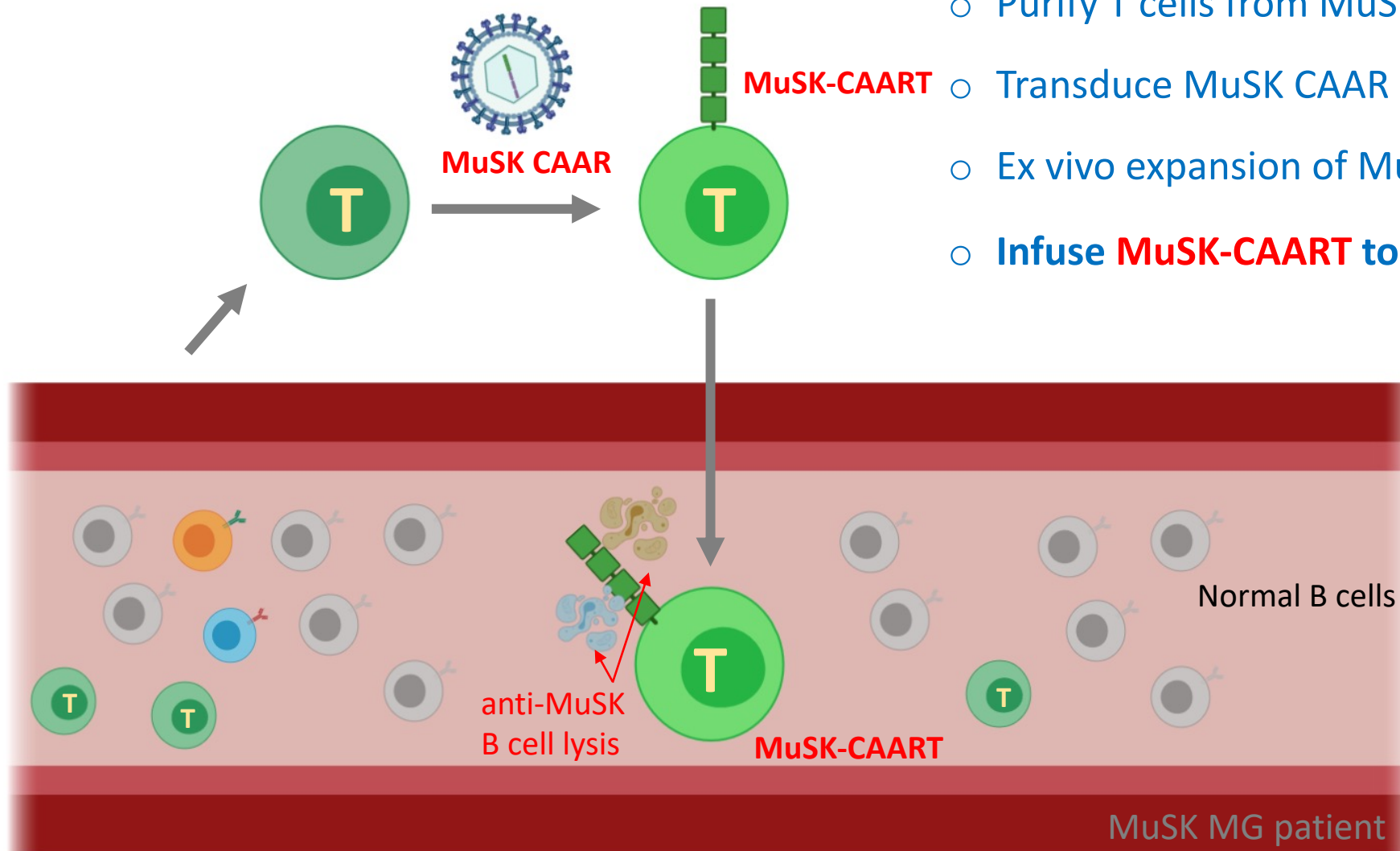
MuSK-CAART is designed to specifically eliminate anti-MuSK B cell receptor (BCR) expressing B cells

- Purify **T cells** from MuSK MG patients
- Transduce **MuSK CAAR** viruses into T cells
- Ex vivo expansion of **MuSK CAAR T cells**

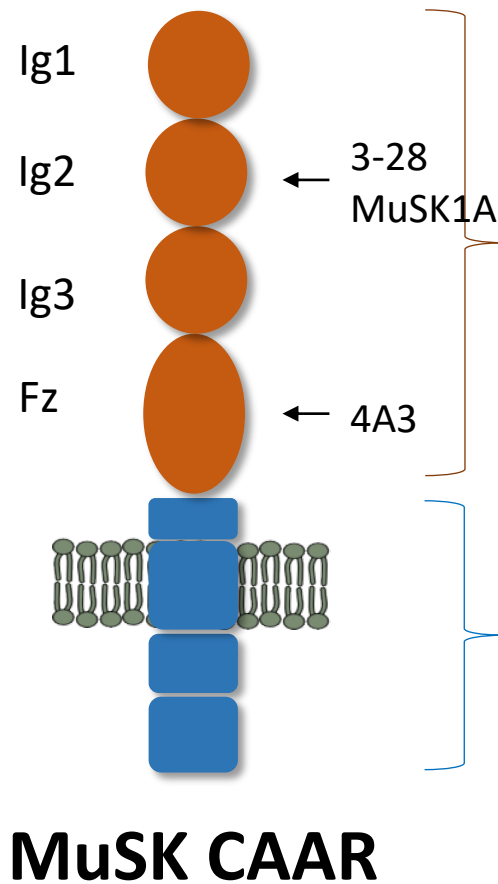


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- Purify T cells from MuSK MG patient
- Transduce MuSK CAAR lentivirus into T cells
- Ex vivo expansion of MuSK CAAR T cells
- Infuse **MuSK-CAART** to MuSK MG patient



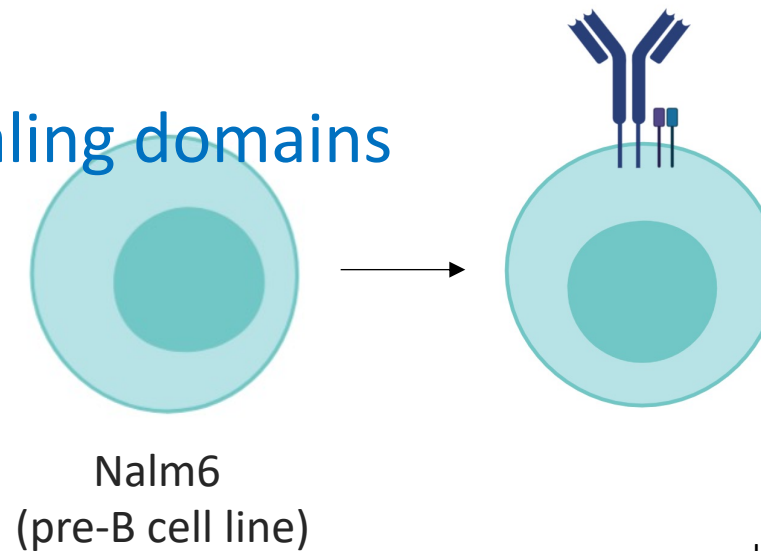
Development of anti-MuSK B cells targeting a variety of MuSK epitopes



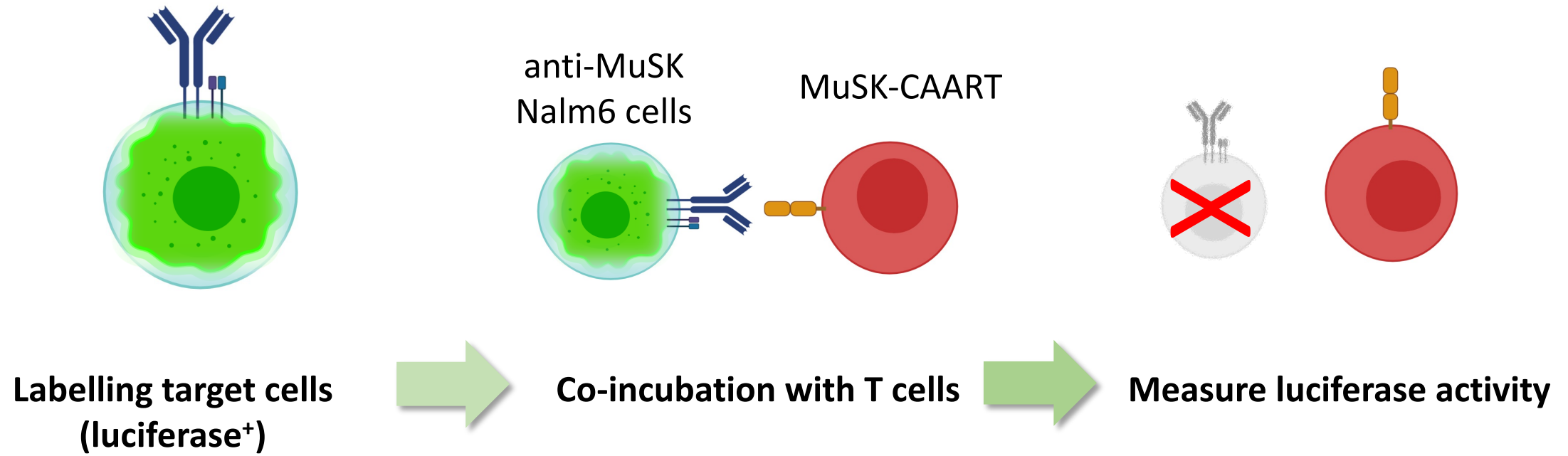
Three different target cells

- 3-28 and MuSK1A were cloned from a MuSK MG patient
- **MuSK extracellular domains** 4A3 was isolated from MuSK-immunized mice
- Affinity: MuSK1A > 4A3 > 3-28
- **Anti-MuSK BCR expressing target cells**

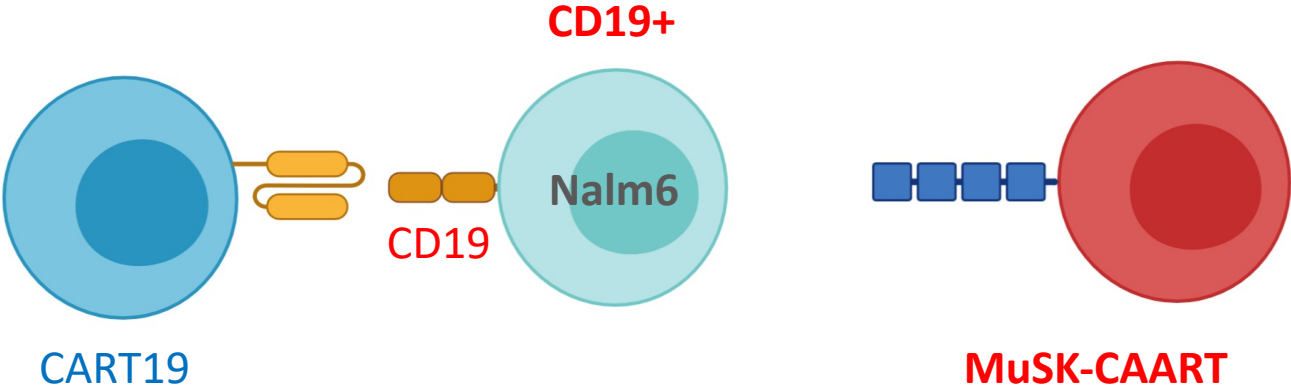
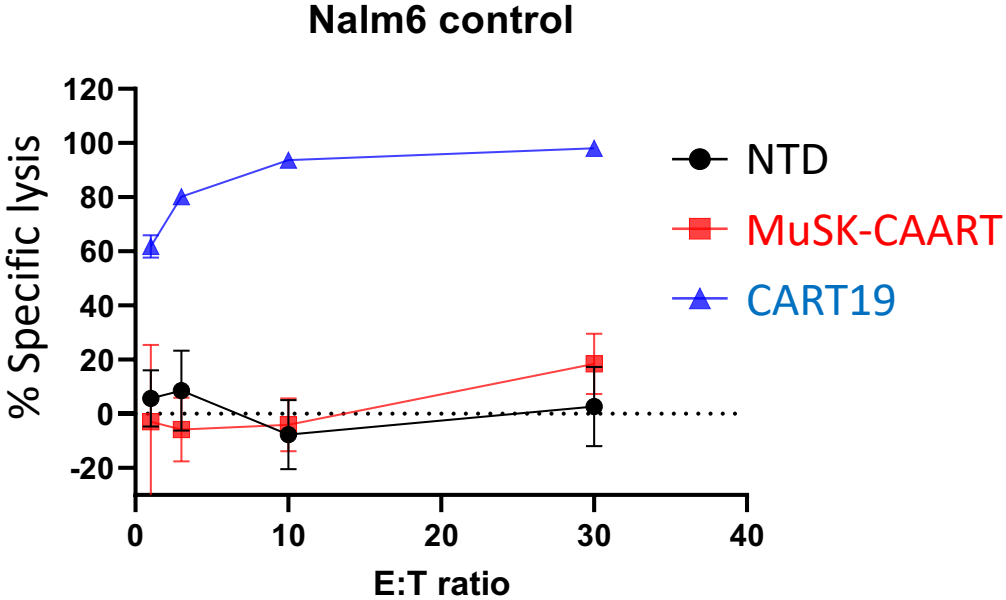
TCR signaling domains



Cytotoxicity of MuSK CAAR T cells against anti-MuSK B cells using a luciferase-based assay

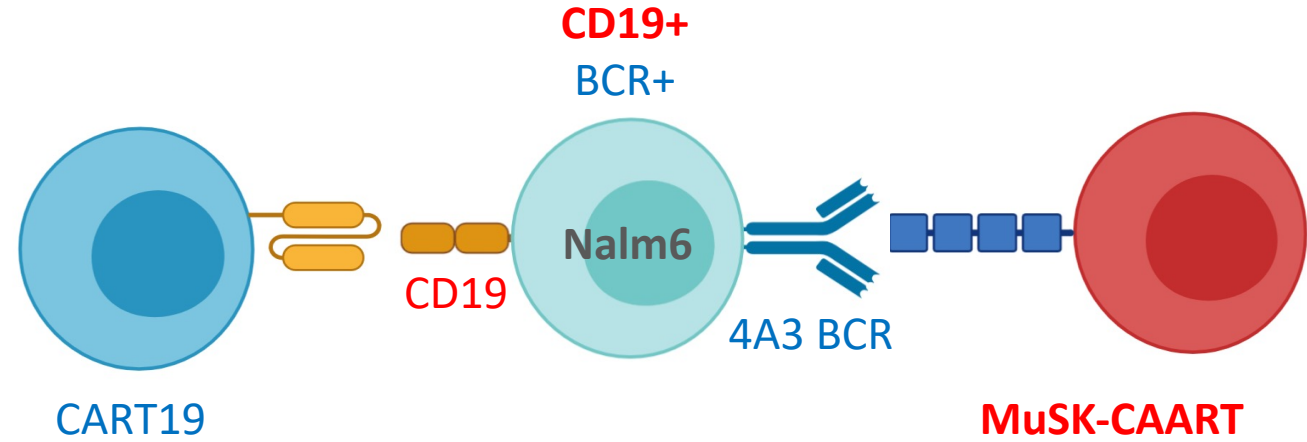
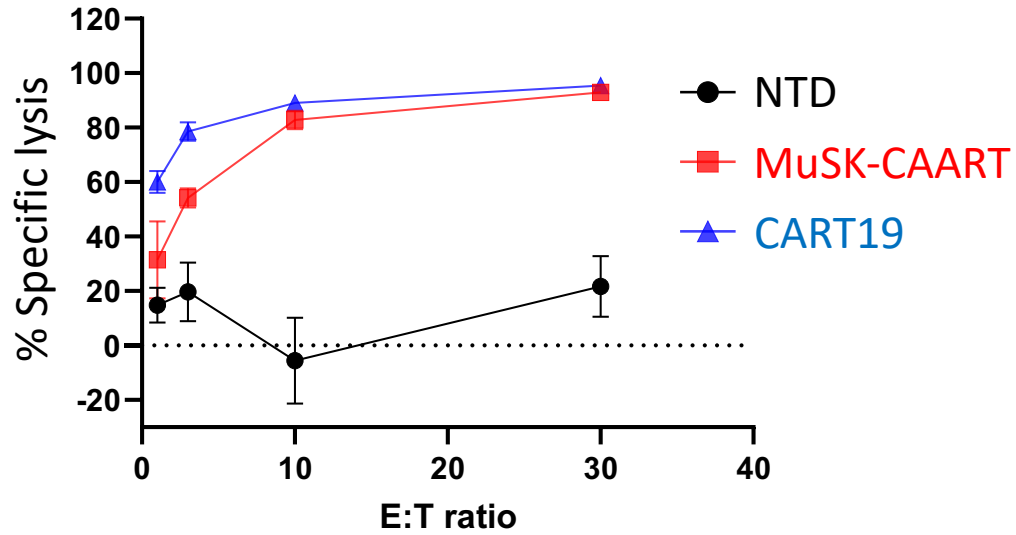


MuSK CAAR T cells do not demonstrate cytotoxicity against control B cells

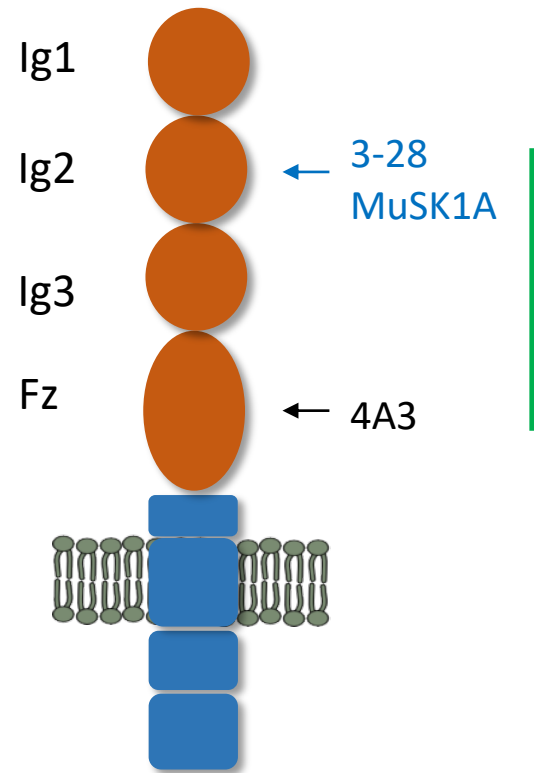


MuSK CAAR T cells effectively kill anti-MuSK B cells

Nalm6 4A3

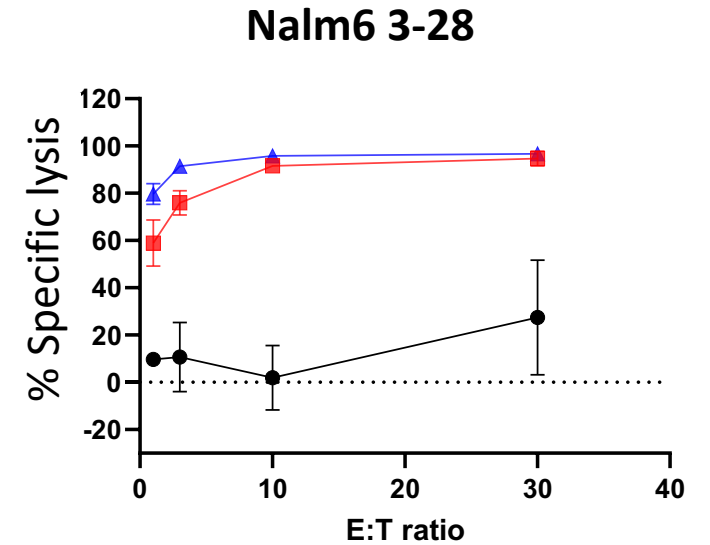
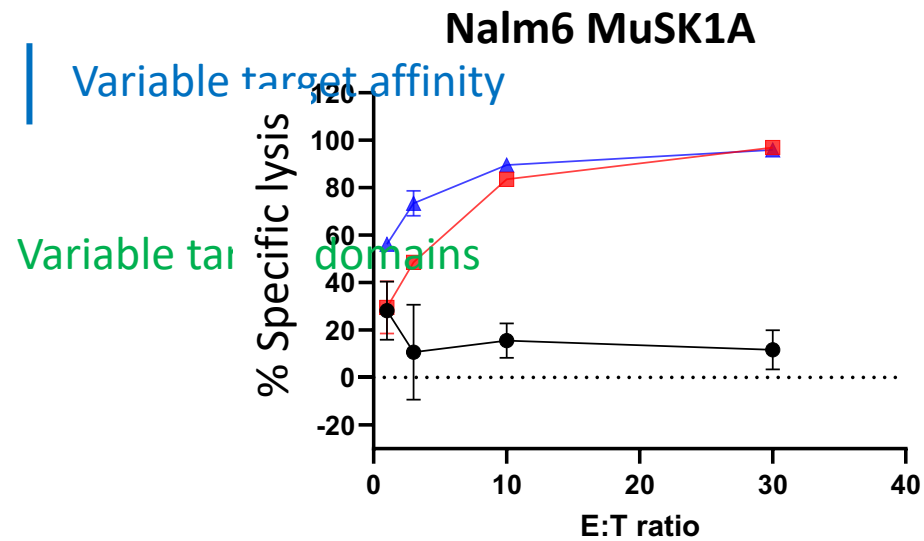


MuSK CAAR T cells kill a range of anti-MuSK BCR expressing target cells



MuSK CAAR

Antibody affinity: MuSK1A > 3-28

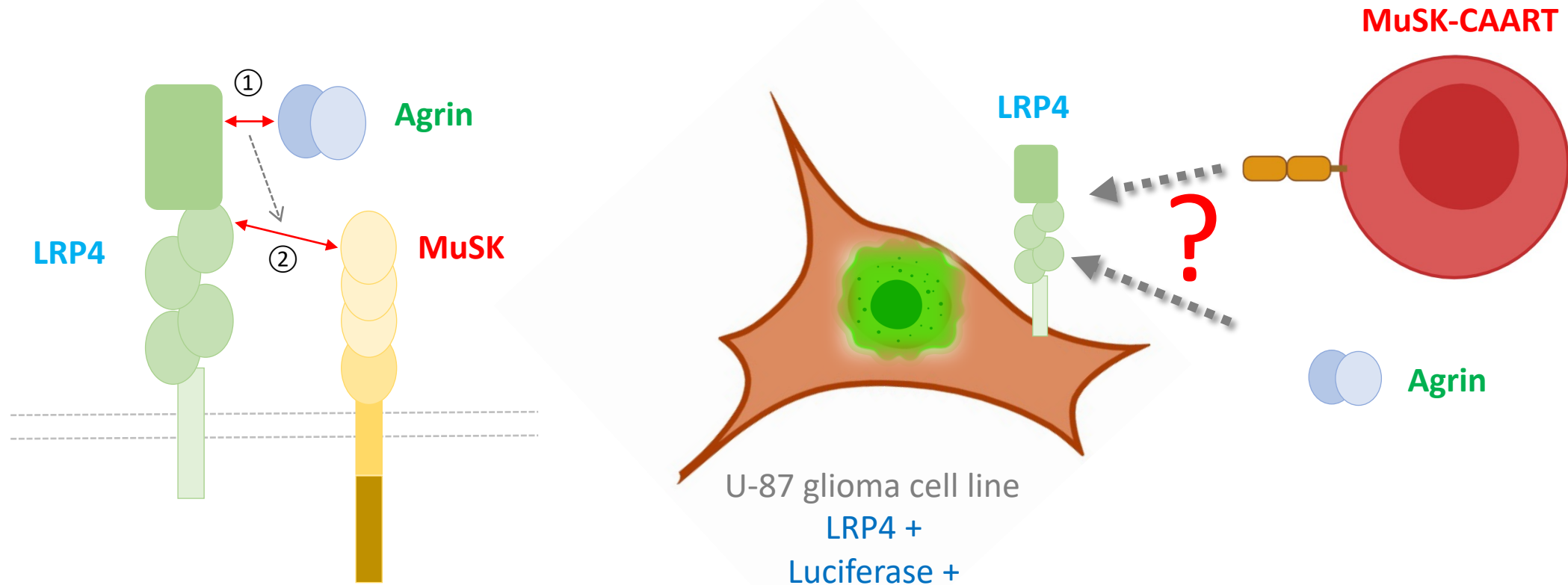


- NTD
- MuSK-CAART
- ▲ CART19

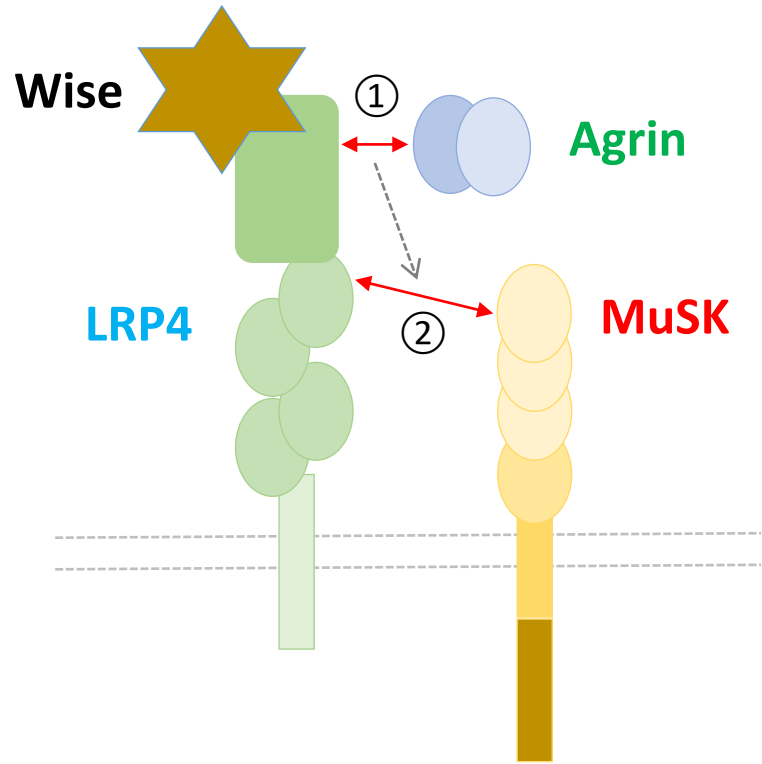


Evaluating potential off-target cytotoxicity of MuSK CAAR T cells

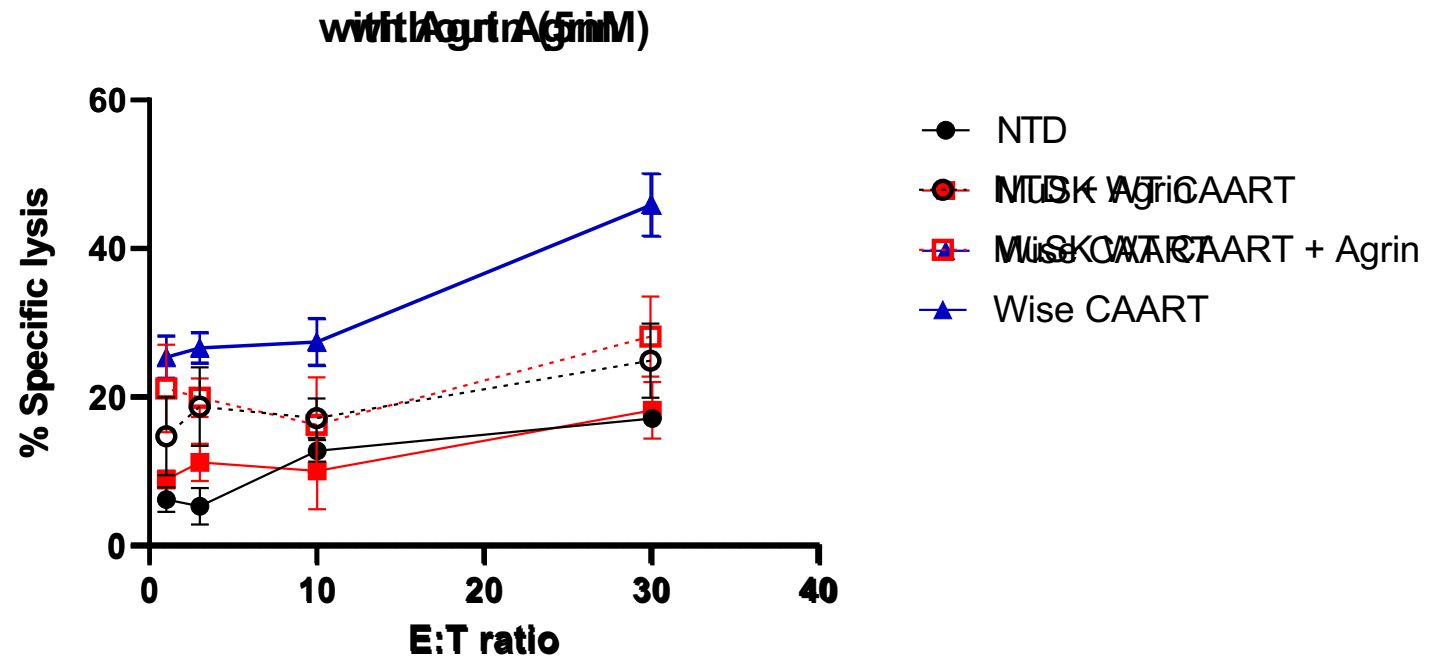
- *LRP4 is a MuSK cis-interacting protein*
- *Agrin-bound LRP4 binds and activates MuSK, which induces AChR clustering*
- *U-87 glioma cells express LRP4*



MuSK CAAR T cells showed no or low off-target toxicity against LRP4 expressing cells

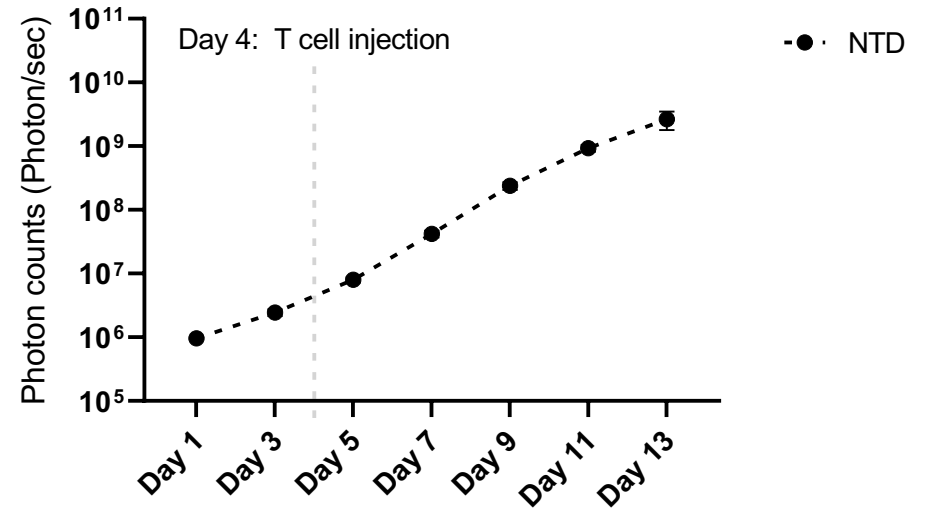
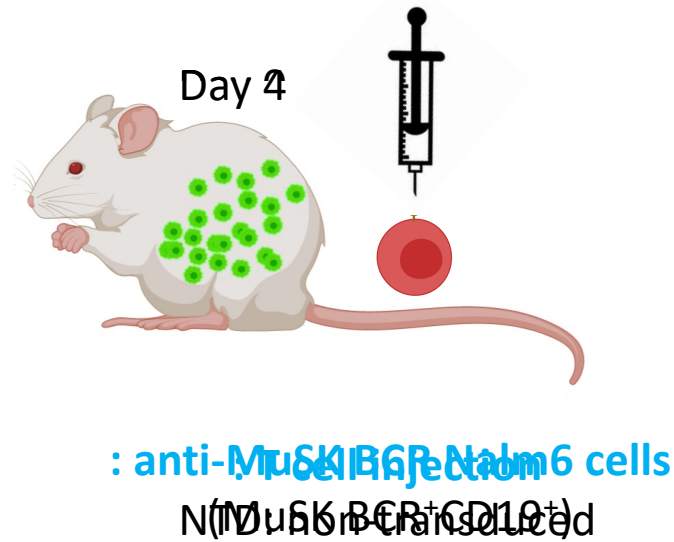
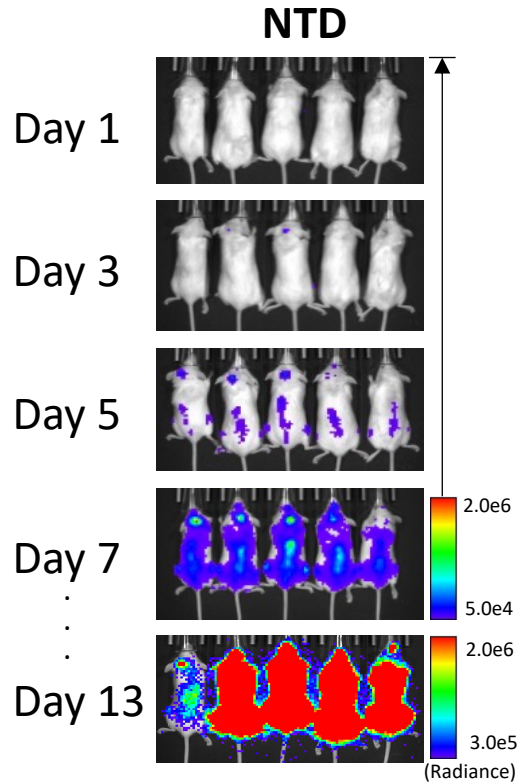


Wise binds LRP4 independent of Agrin



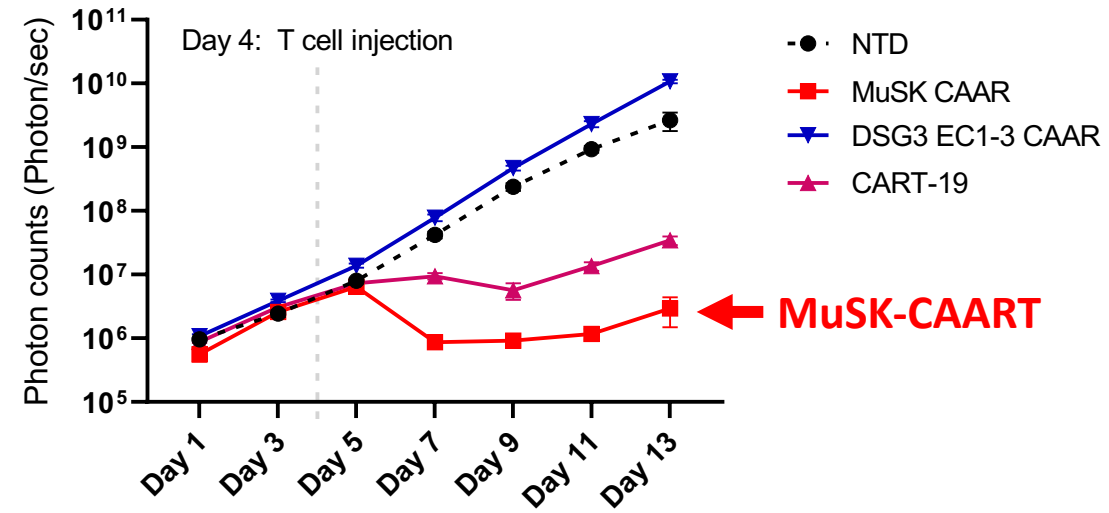
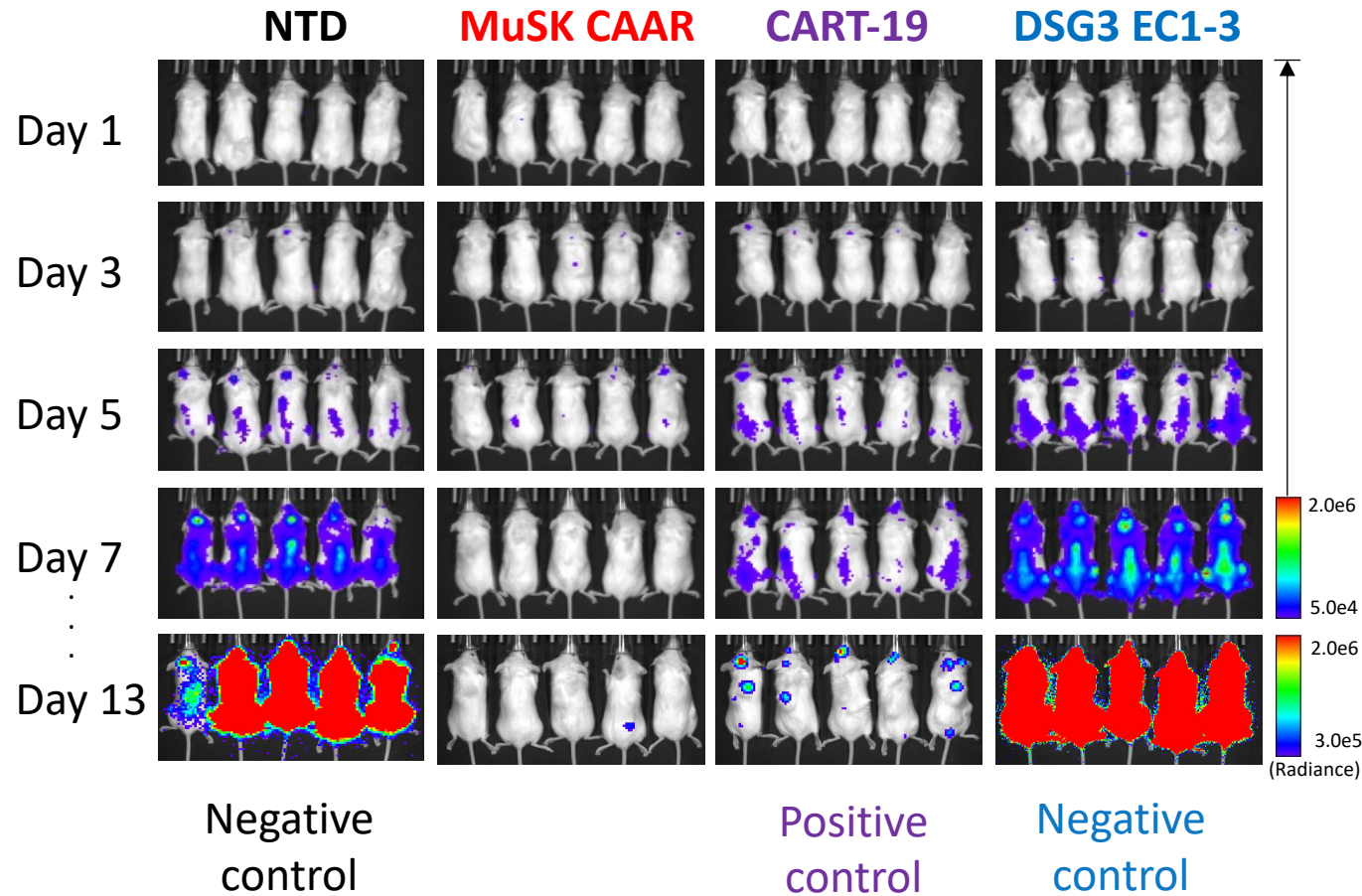
Anti-MuSK BCR expressing Nalm6 engraftment in NSG mice

- *Non-transduced (NTD) T cells were injected at Day+4 after target cell injection*



MuSK-CAART cells and CART-19 cells successfully eliminate anti-MuSK BCR expressing Nalm6 cells

- Target Nalm6 cells express both CD19 and anti-MuSK BCRs

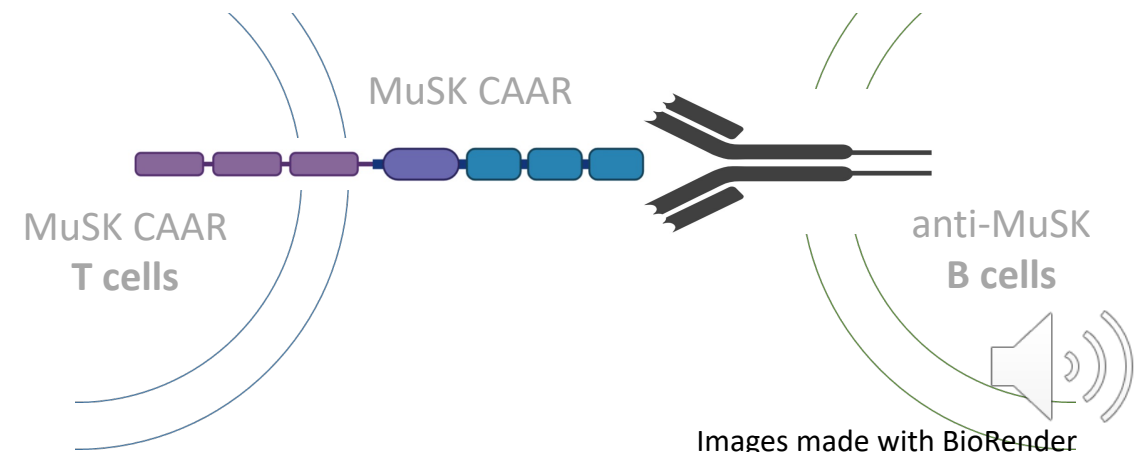


SUMMARY AND FUTURE DIRECTIONS

- MuSK CAAR T cells efficiently kill various anti-MuSK BCR expressing cells but not control B cells
- MuSK CAAR T cells do not show off-target toxicity toward LRP4 expressing cells
- MuSK CAAR T cells efficiently eradicate target cells in NSG mouse model

○ Future Directions

- Further in vitro and in vivo screening for off-target toxicity
- Further investigating animal models to evaluate adoptive human T cell therapy



Images made with BioRender

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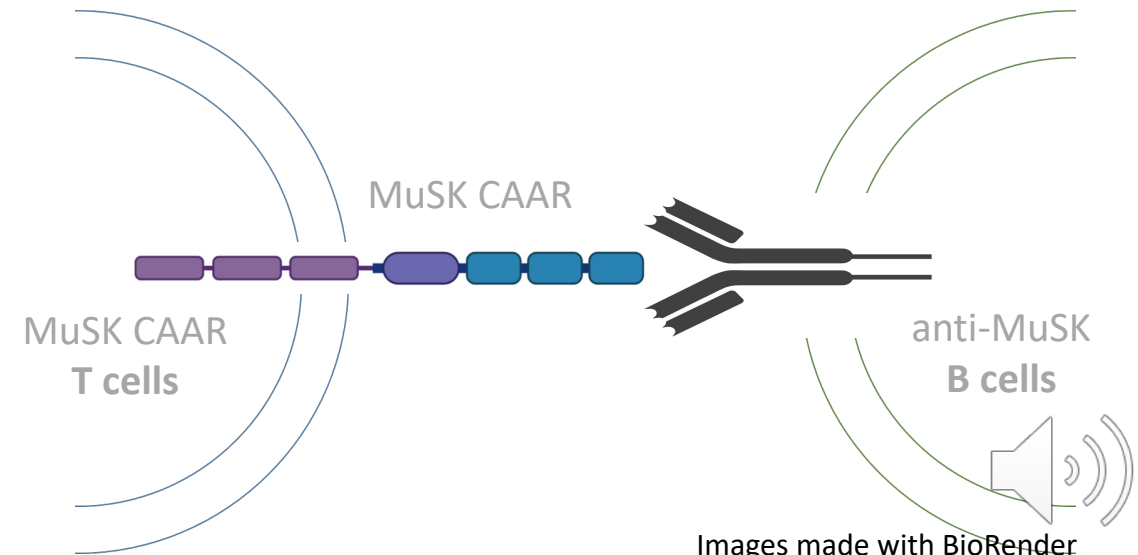
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Thank you!

swoh@pennmedicine.upenn.edu



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