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

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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). Autoreactive B cells produce autoreactive antibodies. Autoantibodies interrupt neuronal signal transduction. Ptosis in a patient with MG (Posey & Spiller, ed. 1904).
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

- **MG patients**
  - **AChR MG** (80-90%)
  - **MuSK MG** (6-7.5%)
  - **SN MG** (LRP4, others) (4-12%)

*Seronegative (SN): anti-AChR & anti-MuSK Ab test negative*
Most treatments for MuSK MG involve off-label immunosuppressants.

**Autoreactive B cells**

- **Prednisone**: global immunosuppressant
- **Mycophenolate mofetil/azathioprine**: preferentially inhibits lymphocytes
- **Rituximab**: anti-CD20 monoclonal antibody targeting 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.
Success of Chimeric Antigen Receptor (CAR) T cell therapy for B cell leukemias and lymphomas

- **scFv**: single-chain variable fragment

**Anti-CD19 scFv**

**CD19**

**Effector T cells**

**B cells**

**FDA approved CAR-T cell therapies**

- Tisagenlecleucel: Aug 30th, 2017
- Axicabtagene ciloleucel: Oct 18th, 2017
Chimeric autoantibody receptor (CAAR) T cells are designed to specifically target antigen specific B cells.

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

Effector T cells

效ector T BCR

Autoreactive B cells

*Pemphigus vulgaris (Blistering skin)*

Images made with BioRender

Ellebrecht et al, Science 2016
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

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

- 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

Images made with BioRender
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
- 4A3 was isolated from MuSK immunized mice
- Affinity: MuSK1A > 4A3 > 3-28
- Anti-MuSK BCR expressing target cells

Images made with BioRender
Takata et al., JCI insight, 2019
Cytotoxicity of MuSK CAAR T cells against anti-MuSK B cells using a luciferase-based assay

Labelling target cells (luciferase*)

Co-incubation with T cells

Measure luciferase activity
MuSK CAAR T cells do not demonstrate cytotoxicity against control B cells.
MuSK CAAR T cells effectively kill anti-MuSK B cells

% Specific lysis

E:T ratio

Nalm6 4A3

-20 0 20 40

0 10 30 40

CD19+ BCR+

CD19

4A3 BCR

CART19

MuSK-CAART

Images made with BioRender
MuSK CAAR T cells kill a range of anti-MuSK BCR expressing target cells

**Antibody affinity:** MuSK1A > 3-28

Variable target domains

Variable target affinity

Nalm6 MuSK1A

Nalm6 3-28

% Specific lysis

E:T ratio

- 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

Images made with BioRender
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
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