Tackling Cancer Cachexia with CAR-Expressing Engineered Macrophages

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Cancer cachexia is a muscle and fat wasting syndrome.

- Cachexia is experienced by 80% of advanced-stage cancer patients and directly causes 30% of cancer-related deaths, yet it is strikingly understudied. [1]
- No treatments exist beyond exercise and diet regimens in the United States. [2]
- Cachexia is primarily caused by systemic inflammation due to the overabundance of interleukin-6 (IL-6) initially produced by the tumor. [3]

Receptor-mediated phagocytosis of IL-6 may ameliorate cachexia.

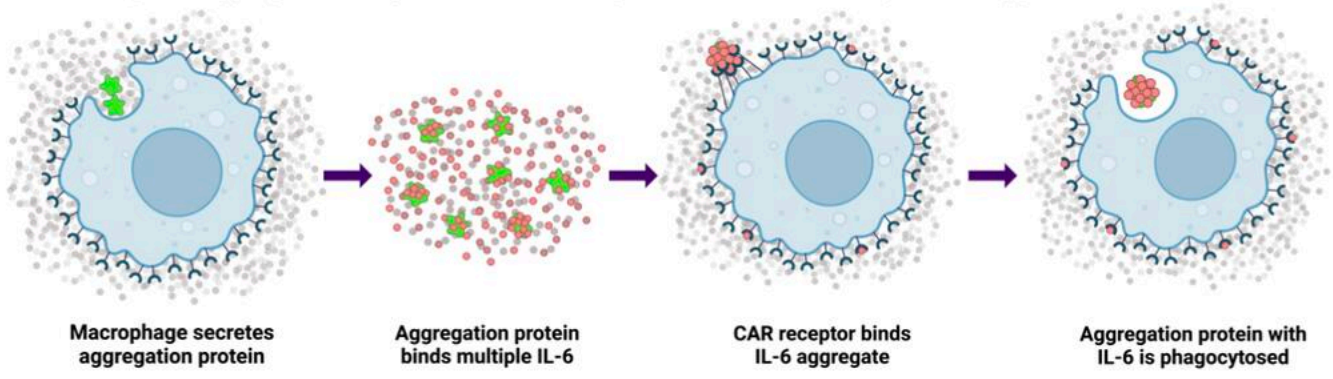
- We are engineering macrophages to recognize IL-6 via a chimeric antigen receptor (CAR) in order to induce phagocytosis.
- Macrophages require a size and signal threshold for phagocytosis; therefore, IL-6 aggregates must be created. [4]

In vivo design:

- Engineering human macrophages to express our CAR and secrete an aggregation protein that binds to multiple IL-6 molecules

In vitro experimentation:

- Transfecting RAW 264.7 murine macrophages with our CAR
- Modeling IL-6 aggregates using anti-FLAG microspheres bound to multiple IL-6 tagged with 3xFLAG



Methods

Experiments:

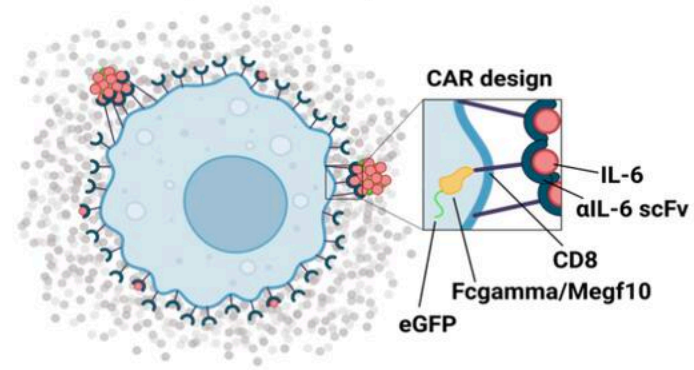
- Transfecting HEK293 cells to produce IL-6 tagged with mCherry and 3xFLAG
- Purifying and binding IL-6 fusion protein to anti FLAG microspheres creating aggregates
- Observing RAW264.7 cells' interaction with IL-6 aggregates

Data collection:

- Fluorescent microscopy to visualize membrane localization of CARs and internalization of IL-6 beads
- Fluorescence-activated cell sorting to isolate successfully transfected cells
- Flow cytometry to quantify CAR expression and IL-6 phagocytosis

Designing a modular CAR

- Our CAR fuses together the single chain variable fragment (scFv) from the anti IL-6 monoclonal antibody *sirukumab* with either an Fcγ or Megf10 intracellular domain to induce phagocytosis.
- We have additionally designed CAR-TK, a **C**himeric **A**ntigen **R**eceptor **T**ool**k**it compatible with the Mammalian Toolkit [5].
- CAR-TK facilitates CAR plasmid construction by allowing for customization of extracellular, spacer, transmembrane, and intracellular parts before assembly into a transcriptional unit.



Progress and ongoing work

- Constructed CAR plasmids using the Mammalian Toolkit
  - Prepared plasmids to test both stable and transient transfection
- Optimized transfection of HEK293 cells successfully and visualized fluorescence of mCherry and eGFP using the fluorescence microscope
- Adjusted gates in the flow cytometer to match our expected fluorescence

Ongoing work:

- Transfecting RAW 264.7 murine macrophages with CARs
- Assaying phagocytic activity via microsphere experiments
- Incorporating genetic circuitry to selectively modulate IL-6 levels and localize to the tumor microenvironment
- Designing the aggregation protein
- Developing a combinatorial screen using the CAR-TK concept

References

[1] "Treating Cancer Cachexia: Progress Looks Possible." National Cancer Institute, www.cancer.gov/about-cancer/treatment/research/cachexia.
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[3] Narsale, Aditi A., and James A. Carson. "Role of Interleukin-6 in Cachexia." Current Opinion in Supportive & Palliative Care, vol. 8, no. 4, 2014, pp. 321 –327, https://doi.org/10.1097/spc.0000000000000091.
[4] Champion, Julie A., et al. "Role of Particle Size in Phagocytosis of Polymeric Microspheres." Pharmaceutical Research, vol. 25, no. 8, 2008, pp. 1815–1821, https://doi.org/10.1007/s11095-008-9562-y.
[5] Assembly of Genetic Circuits with the Mammalian ToolKit. Fonseca JP, Bonny AR, Town J, El-Samad H. Bio Protoc. 2020 Mar 5;10(5):e3547. doi: 10.21769/BioProtoc.3547. PubMed: PMID: 33659521 .Assembly of Genetic Circuits with the Mammalian ToolKit. Fonseca JP, Bonny AR, Town J, El-Samad H. Bio Protoc. 2020 Mar 5;10(5):e3547. doi: 10.21769/BioProtoc.3547. PubMed: PMID: 33659521 .