The Use of Genetically Engineered Macrophages to Combat the Tumor Microenvironment

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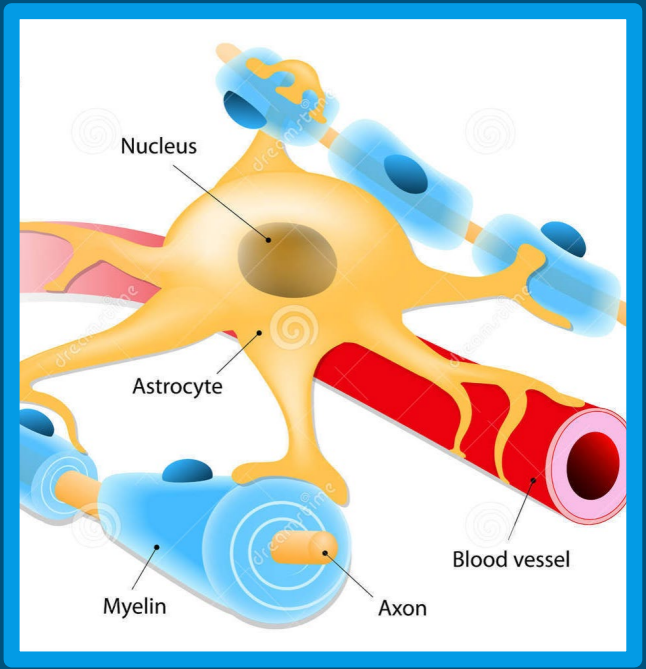
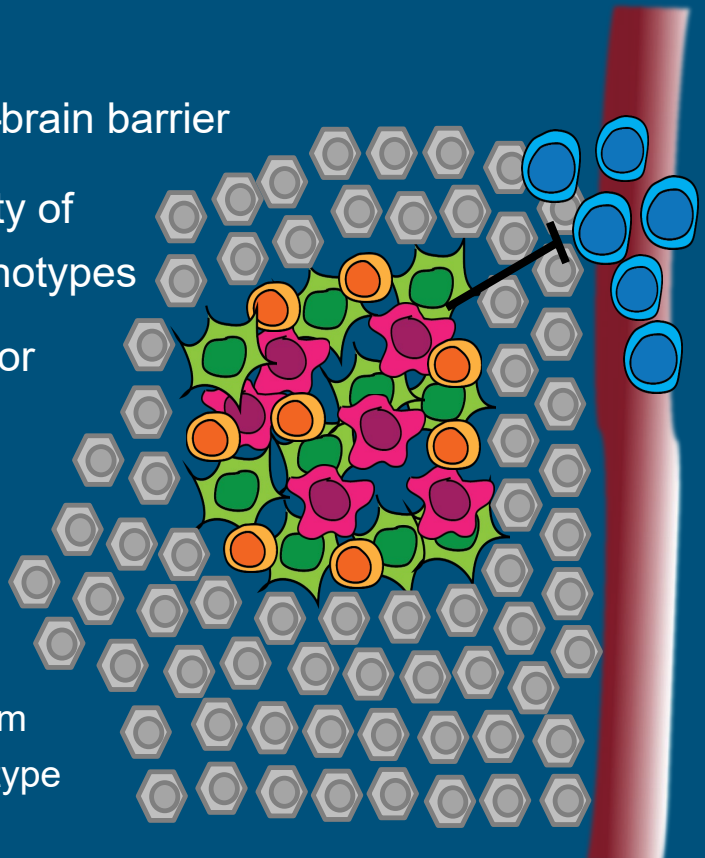
Glioblastoma (GBM)



- GBM is a fast-growing, highly aggressive brain tumor
  - WHO Grade IV Astrocytoma
  - Median survival from diagnosis is 14.6 months
- First-line therapies include surgery, chemo, and radiation
  - Side effects significantly reduce quality of life
  - Recurrence
- This makes GBM patients good candidates for immunotherapy
  - Enhances the patient's natural immune function
  - Has the potential to target cancer cells specifically and leave healthy cells intact

Barriers for Immunotherapy in Solid Tumors

- Difficulty in crossing the blood -brain barrier
- Challenges due to heterogeneity of tumor cells having varying phenotypes
- Immunosuppression of the tumor microenvironment (TME)
  - Recruitment of pro-tumor, anti-inflammatory cells
  - Secretion of pro-tumor, anti-inflammatory cytokines
  - Modification of immune cells from pro- to anti-inflammatory phenotype



Normal Cells

Cancer cells

Pro-tumor myeloid cells
- Tumor-associated macrophages

Regulatory immune cells
- T-regs

Cytotoxic immune cells
- NK Cells
- T-Cells

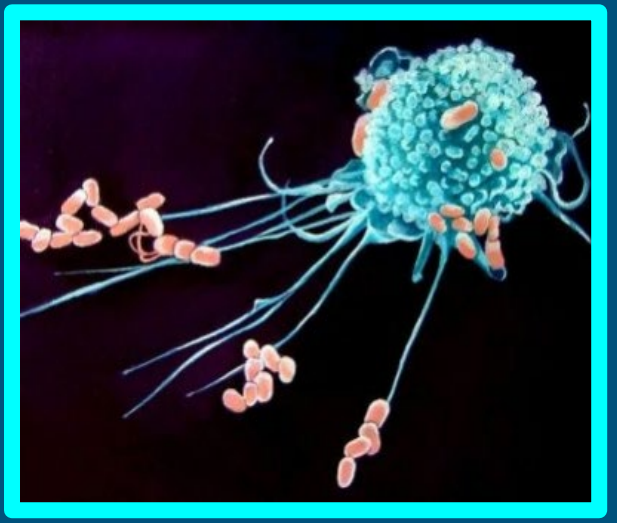
Goals for Cancer Treatment

Goal: Eliminate the immunosuppressive factors of the tumor microenvironment and facilitate the destruction of cancerous cells by enhancing the cytotoxic immune response

Our Approach: Genetically engineer macrophages to express factors that could boost anti -tumor immune activity

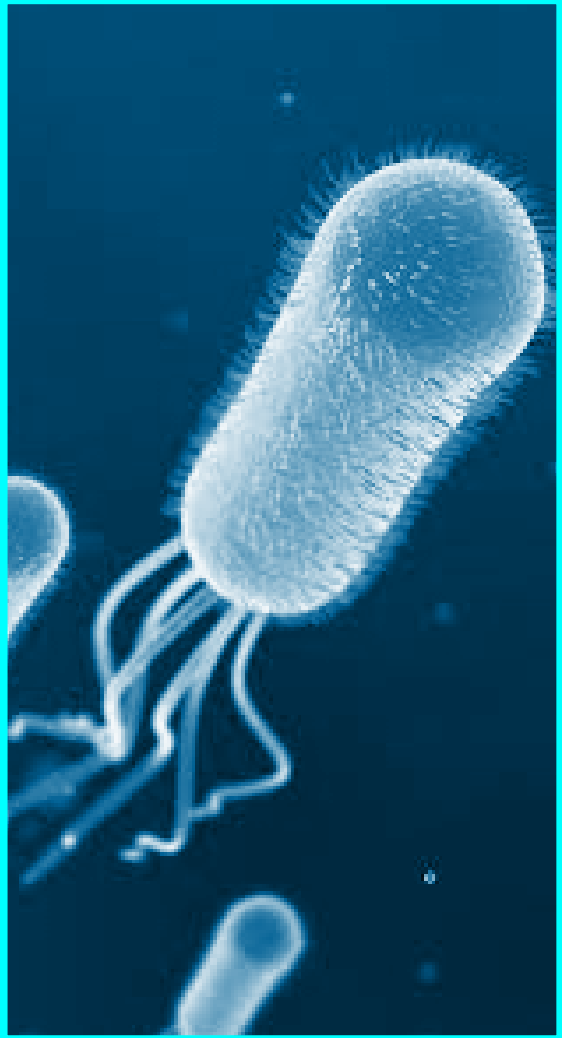
Genetically Engineered Macrophages (GEMs)

- Macrophages are part of a group of immune cells that act as the first -line of defense against foreign pathogens
  - Pro-inflammatory vs tumor -associated
  - Capable of signaling to cytotoxic cells such as NK and T cells
- GEMs are macrophages that have been transduced with lentivirus, which allows stable protein expression
- Using animal models, the Crane lab has shown GEMs can be directly implanted into the tumor and retained for at least one month
- We hypothesize that GEMs could be used to reprogram the TME in a way that supports immune-mediated tumor destruction



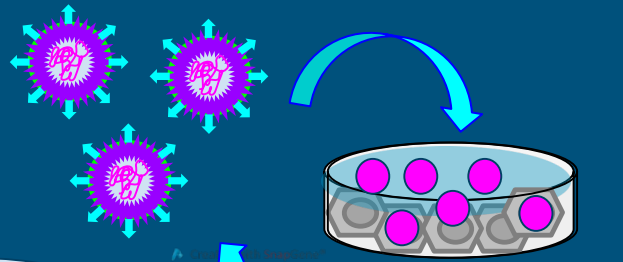
Using a TLR-5 Agonist

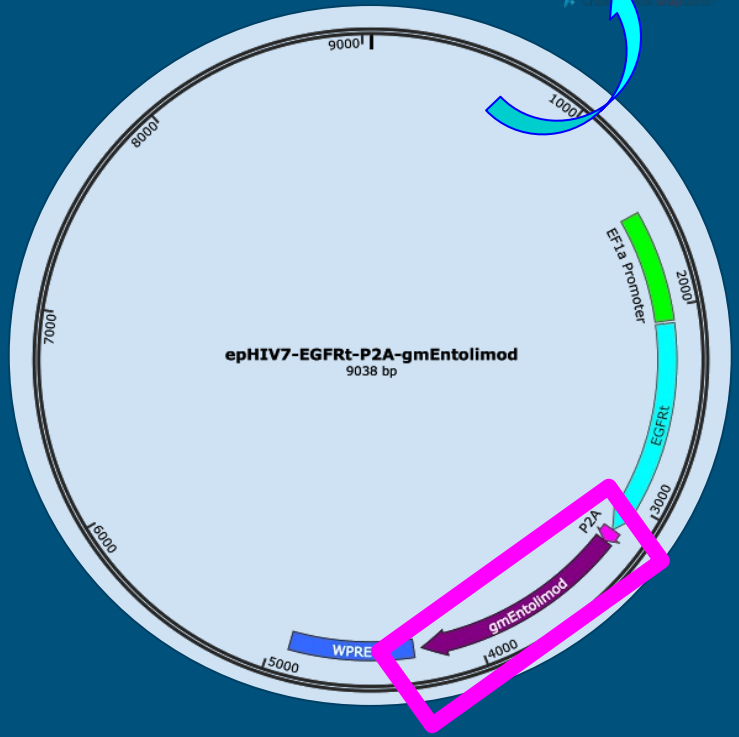
- TLR-5 is a human protein expressed on macrophages
  - Activation of this protein by bacterial flagellin alerts the immune system
- We can create a protein that mimics the segment of flagellin that binds the receptor
  - We anticipate that agonizing this receptor on tumor-associated macrophages will trick them into recruiting anti-tumor immune cells such as NK and T-Cells
- By engineering GEMs to express this TLR-5 agonist, we expect to be able to reprogram the TME



Step One

Clone vector

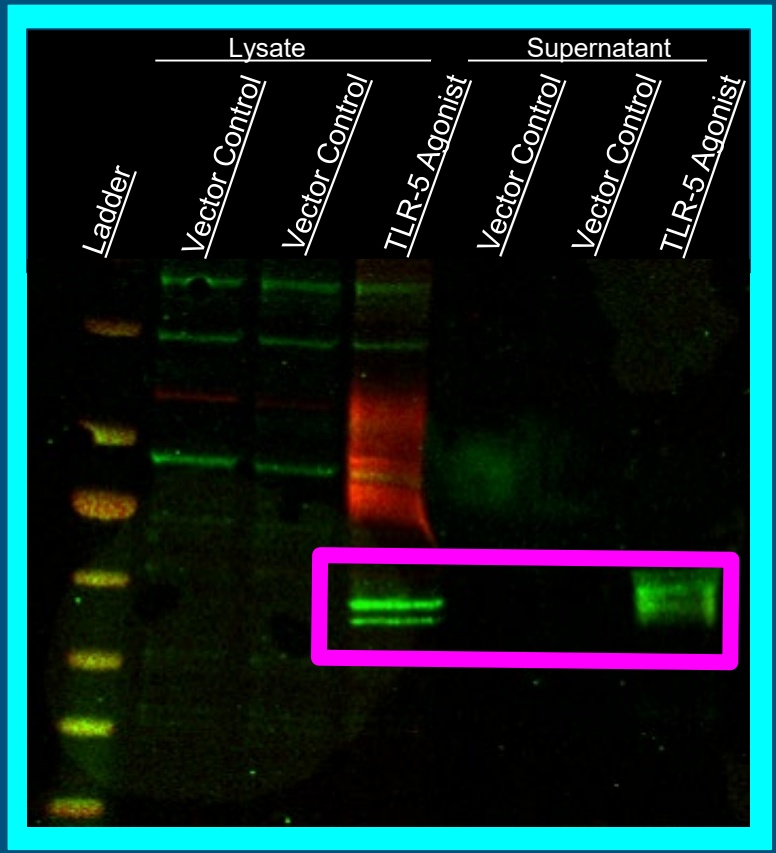




Entolimod=TLR-5 Agonist

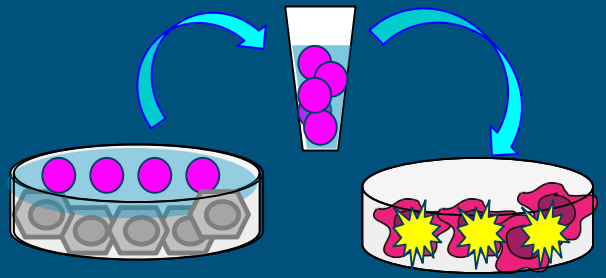
Step Two

Confirm expression in mammalian cells



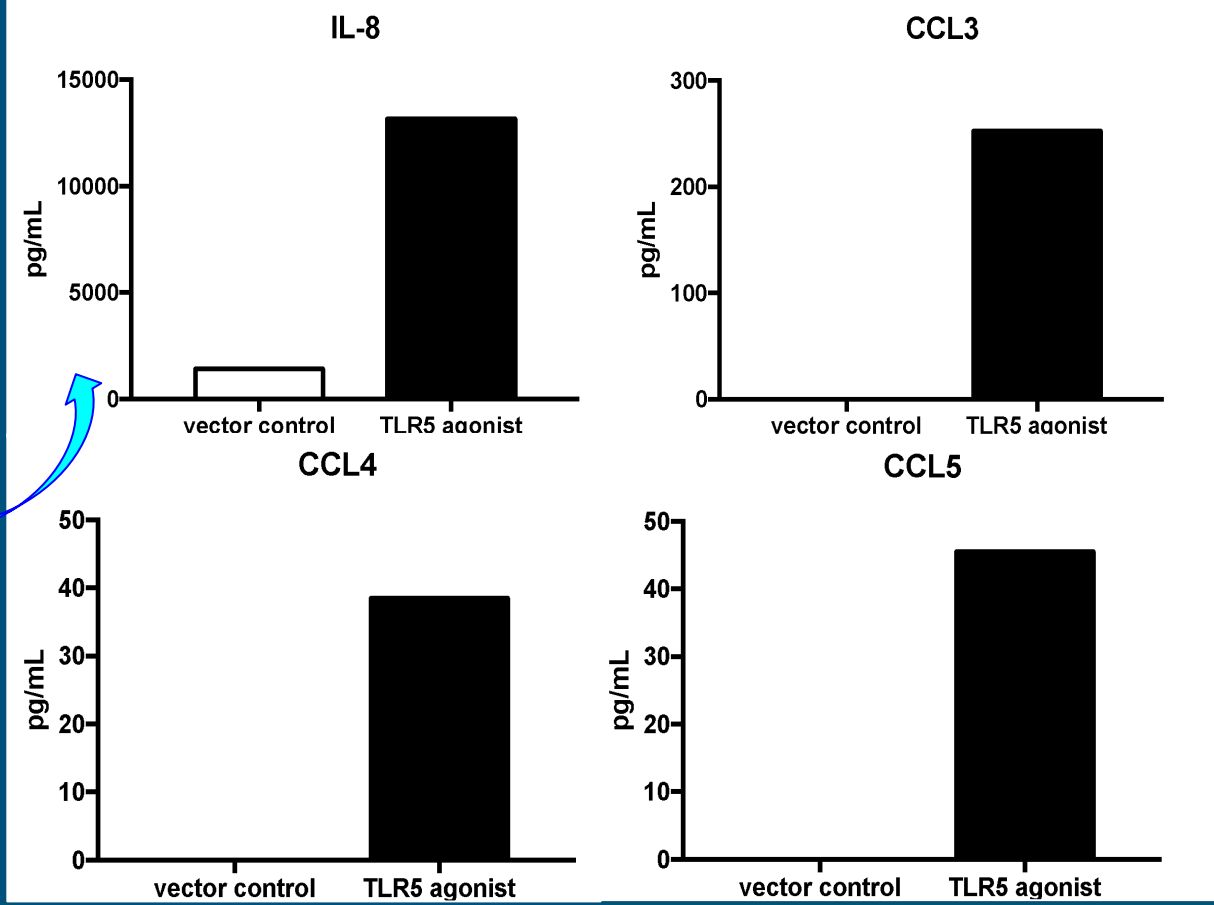
Step 3

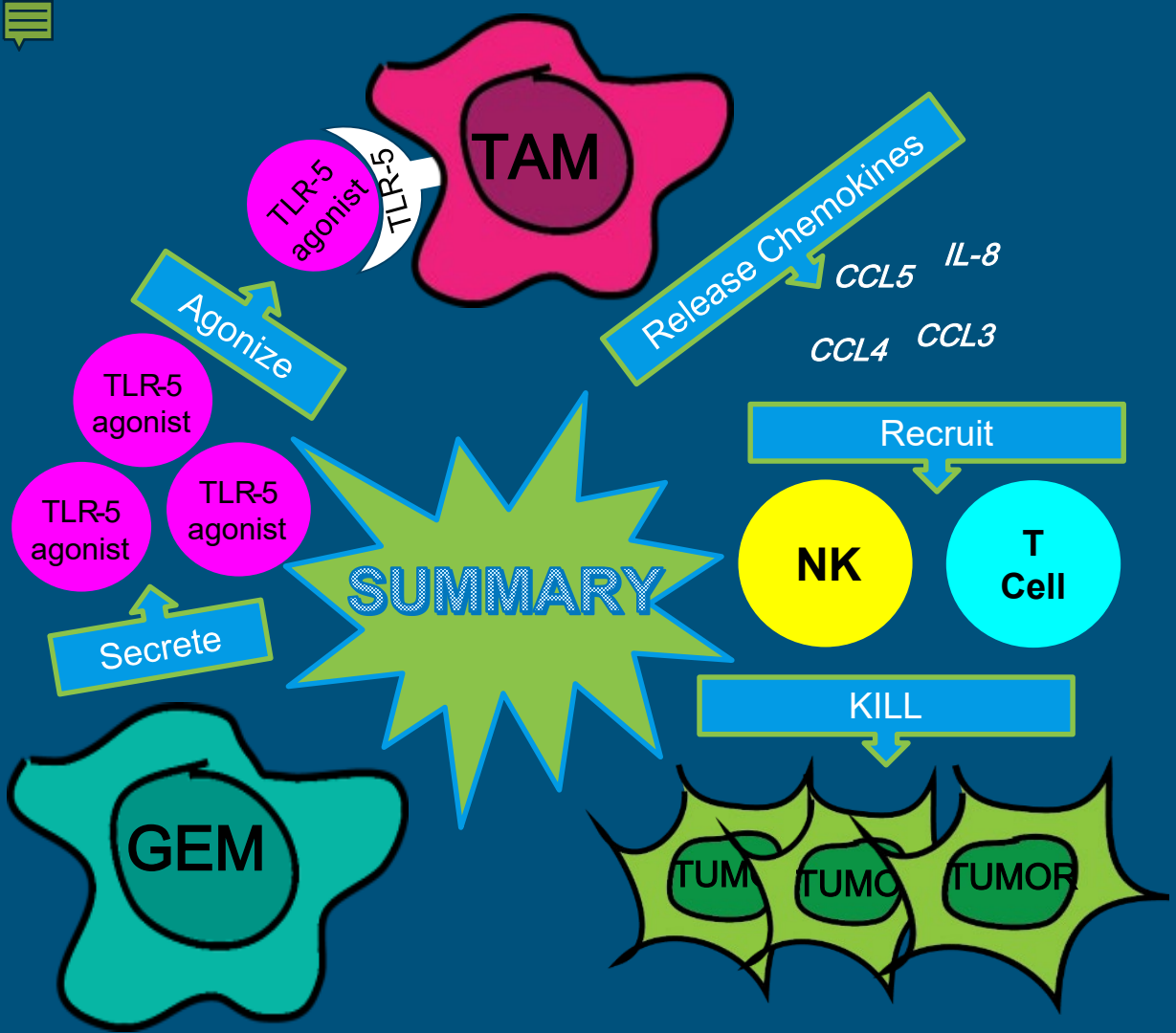
Evaluate macrophage response to TLR-5 agonist



TLR-5 agonist:

- biologically active
- upregulates chemokines known to recruit antitumor immune cells





Conclusion

We expect that stimulation of macrophages with TLR-5 agonist will recruit cytotoxic immune cells to the TME

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