TRANSDUCED MACROPHAGES AS A SOURCE OF NK CELL ACTIVATION AGAINST TUMORS

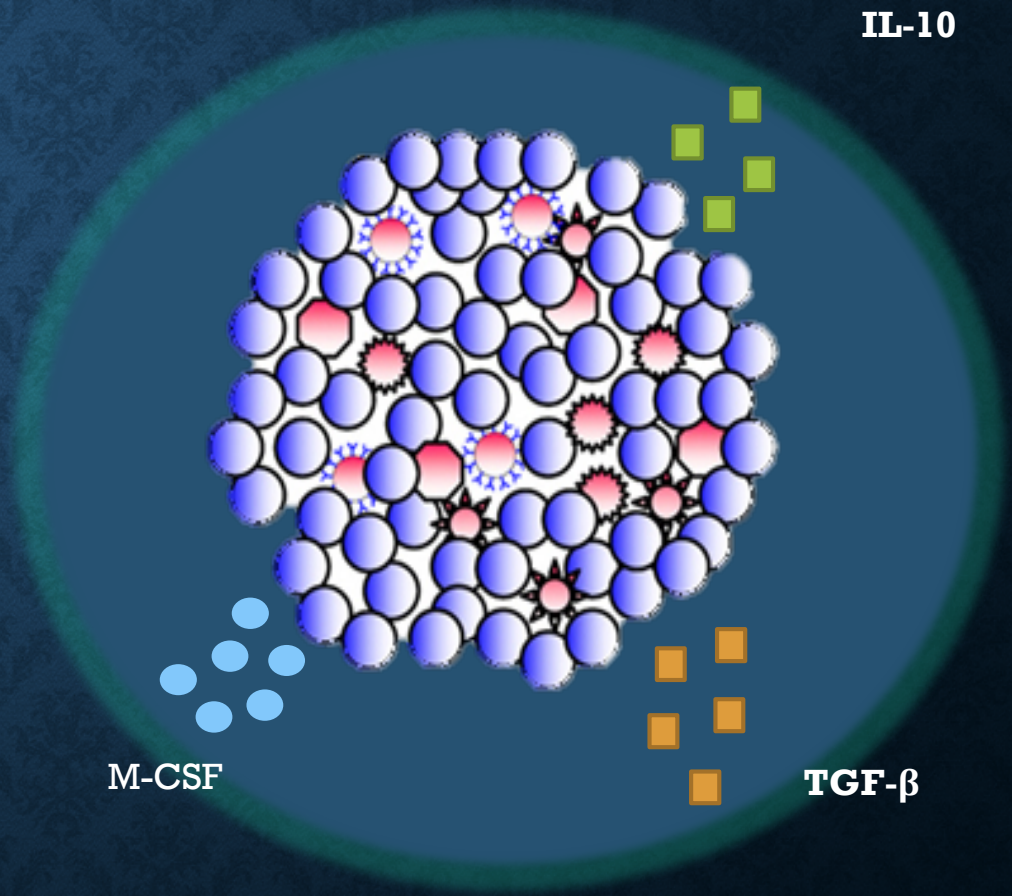
Max Hanson, Student Intern UW Neurological Surgery Summer Student Program

PI: Dr. Courtney Crane, PhD
Crane Lab
Ben Towne Center for Childhood Cancer Research

NIH NINDS R25NS095377 - Summer Research Experience in Translational Neuroscience and Neurological Surgery

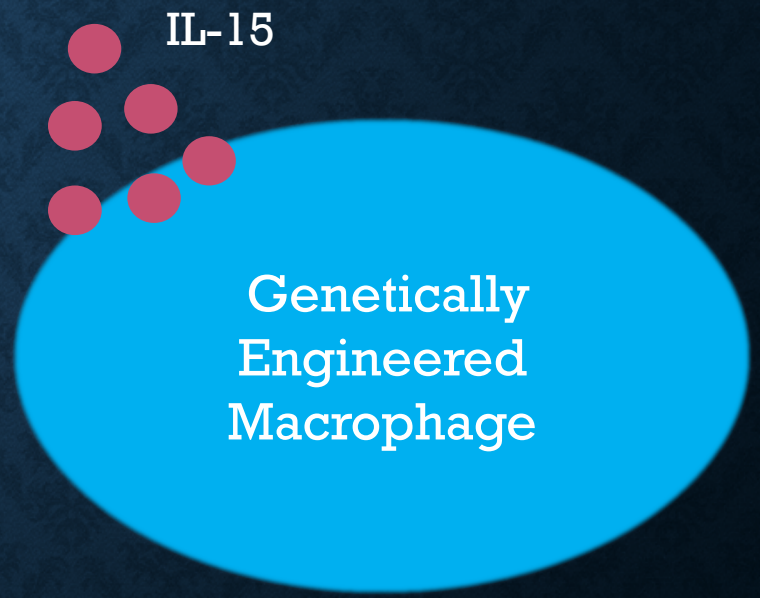
CHALLENGES OF DEVELOPING THERAPEUTICS AGAINST SOLID TUMORS

1. Heterogeneity of tumor cells
   - Not all tumor cells express the same protein targets
2. Tumor microenvironment (TME) suppresses protective immune functions
   - Immunosuppressive cytokines
   - Recruit anti-inflammatory immune cells (Tumor Associated Macrophages)



GENETICALLY ENGINEERED MACROPHAGES (GEMS)

- Macrophages are innate cells important for…
  - Cross talk
  - Pro-inflammatory signals (immune cell recruitment)
- ideal therapeutic cell type
  - Survive in the brain without impacting survival
  - Don't proliferate (inject GEMs into tumor)
  - Can stably express lentivirally transduced DNA (GEMs)
- GEMs can be engineered to secrete immune activating proteins



Given the lack of immune activation within the TME, can we use our GEMs to stimulate an anti-tumor response?

NATURAL KILLER CELLS AND IL-15

- Natural Killer (NK) cells: Cytotoxic immune cells with an anti-tumor response
- Problem: NKs by themselves have a significantly decreased response to **solid** tumors
- sIL-15: immune activating cytokine that has been shown to positively impact NK cell killing

If we give our GEMs DNA coding for sIL-15, will they become a hub for NK cell activation?

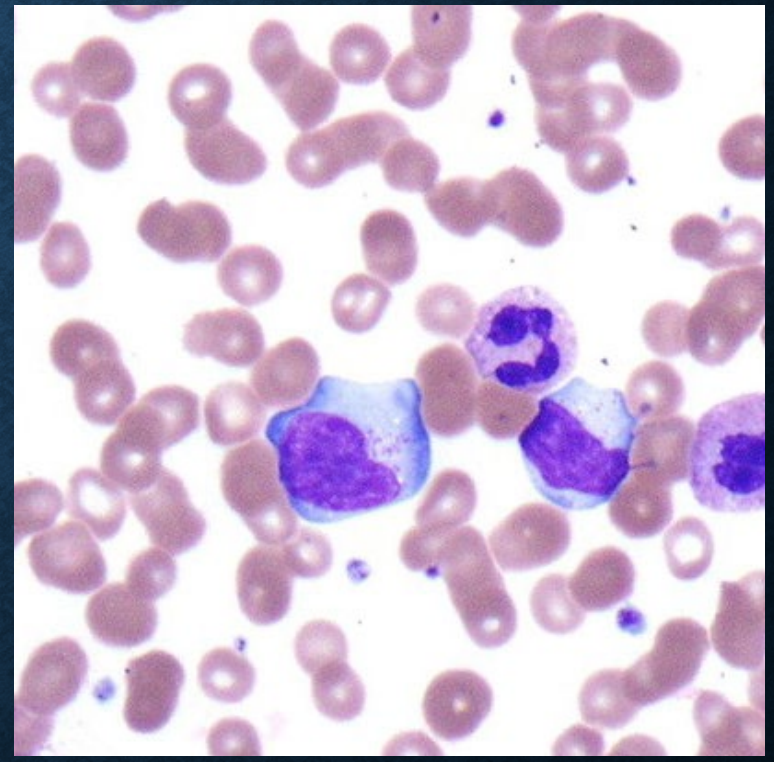
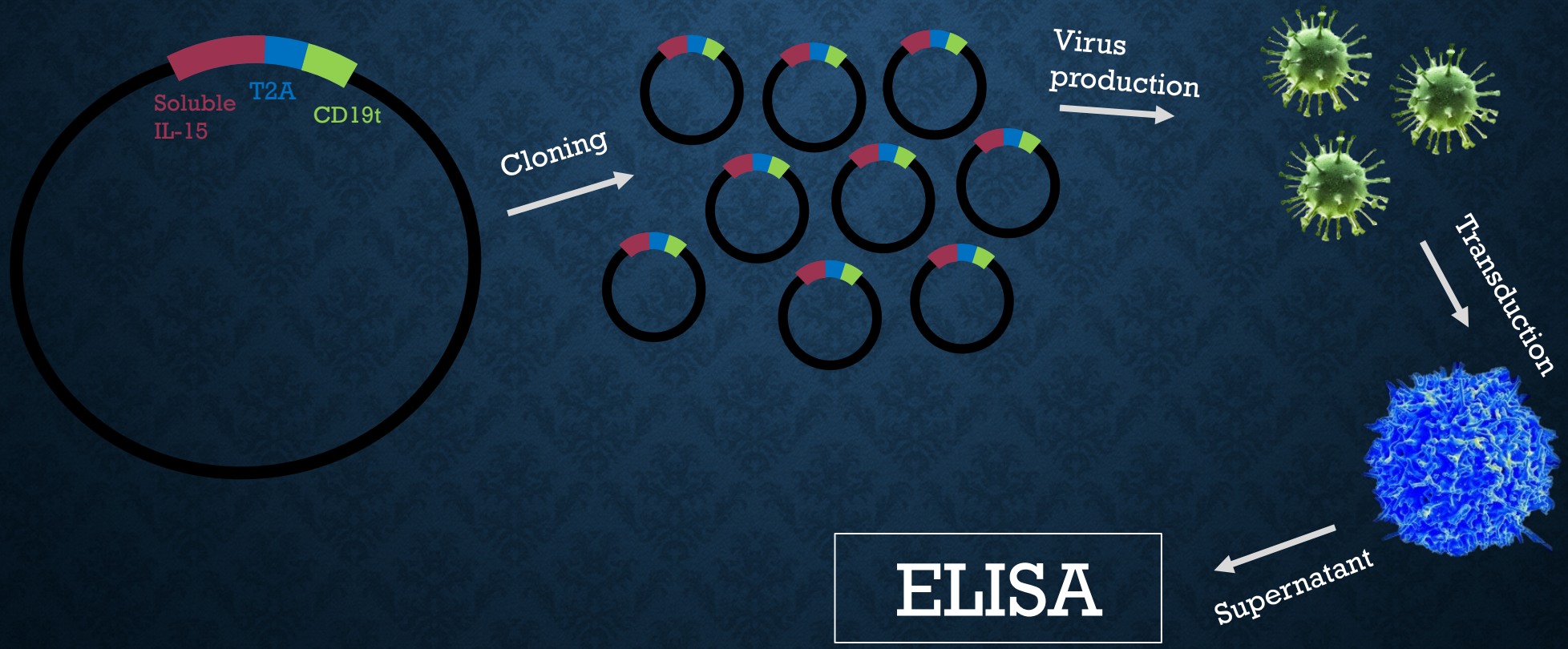


Image from American Society of Hematology Image Bank

OBJECTIVE & METHODS

- First step: transduce macrophages with sIL-15 DNA via lentiviral transduction, and confirm that the transduced GEMs express the cytokine

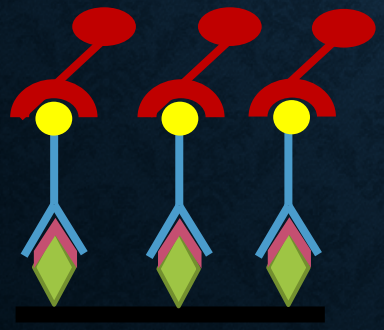


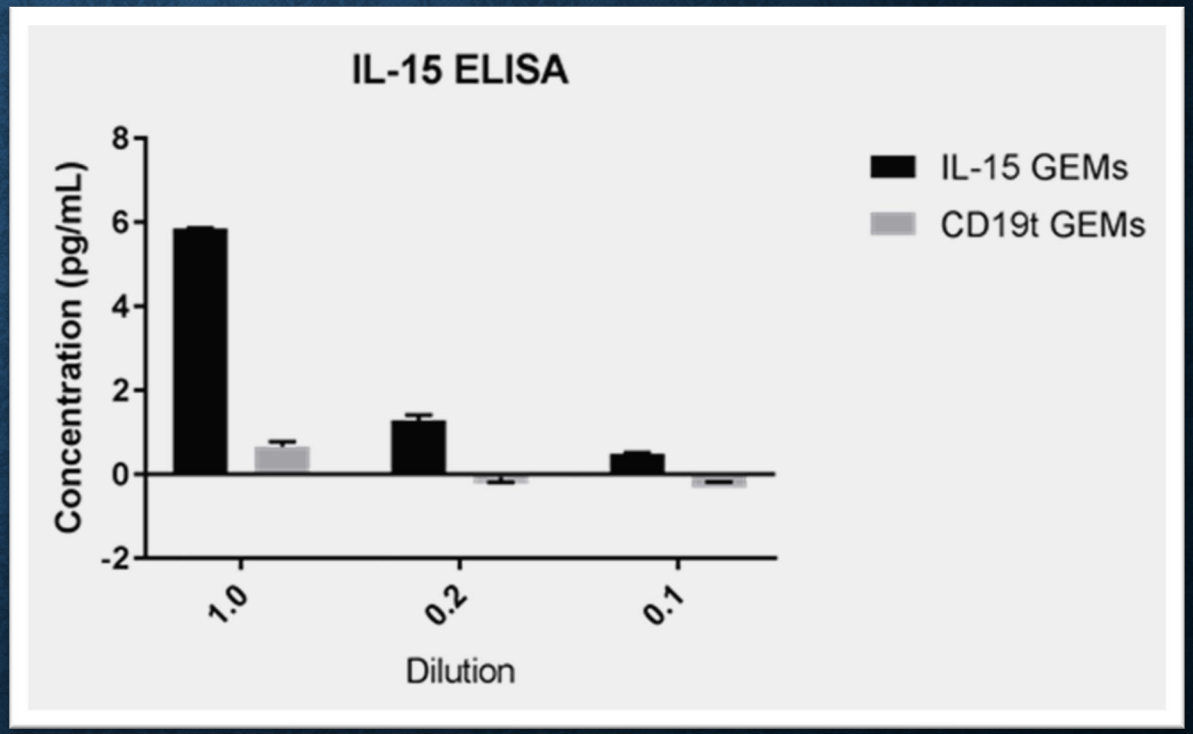
ELISA

Mouse Anti-Human IL-15 antibody on surface attached to IL-15 sample

Biotinylated mIL-15 attached to IL-15 sample

Streptavidin-HRP (enzyme) binds to biotin, create color change





NEXT STEPS

How do our sIL-15 secreting GEMs affect NK cells in vitro?

- Perform a Chromium Release Assay (CRA) to test cytotoxic activity of NK cells after co-culture with sIL-15 GEMs

Effect of sIL-15 GEMs on NK cells in vivo?

- Mice with luciferase labeled tumors receive sIL-15 GEMs and NK cells. Look for smaller amounts of luminosity

THANK YOU

UW Neurological Surgery Summer Student Program

- Dr. and Mrs. Ellenbogen
- Jim Pridgeon
- Dontay Smith

Crane Lab

- Courtney Crane, PhD
- Katie Brempelis, Research Sci III
- Shannon Kreuser, Research Sci I
- Nicole Lieberman, PhD, Post-Doc
- Harrison Chinn, Research Tech I
- Kara White, PhD, Research Sci IV
- Stephanie Balcaitis, Lab Supervisor
- Amira Davis, Lab Research Coordinator
- Kole Degolier, Research Sci I
- Jen Gardell Research III

REFERENCES

- *Amaxa Nuclofector Technology (PDF).* Lonza Cologne AG. Cologne, Germany. 2009. Web.
- Cheng, Min. et al. *NK cell-based immunotherapy for malignant diseases*. Cellular and Molecular Immunology, CSI and USTC, 2013.
- Cooper, Megan. et al. *The biology of human natural killer-cell subsets*. TRENDS in Immunology Vol. 22 No. 11, November 2001.
- Crane, Courtney. et al. *TGF-β downregulates the activating receptor NKG2D and $CD8^+$ T cells in glioma patients.* Department of Neurological Surgery, University of California-San Francisco. San Francisco, CA. Neuro-Oncology. 20o9.
- Dunn, Gavin. et al. *The Immunobiology of Cancer Immunosurveillance and Immunoediting*. Department of Pathology and Immunology/Center for Immunology, Washington University School of Medicine. August, 2004.
- Moyes, Kara. et al. *Genetically Engineered Macrophages: A Novel Platform for Cancer Immunotherapy*. Human Gene Therapt, 19 May, 2016.
- Oleg Tolamachov, Tanya Tolmachova and Faisal A. Al-Allaf (2011). Designing Lentiviral Gene Vectors, Viral Gene Therapy, Dr. Ke Xu (Ed.), ISBN: 978-953-307-539-6, InTech, Available from: http://www.intechopen.com/books/viral-gene-therapy/ designing-lentiviral-gene-vectors
- van Ostaijen-ten Dam, Monique. et al. *Preparation of Cytokine-activated NK cells for Use in Adoptive Cell Therapy in Cancer Patients: Protocol Optimization and Therapeutic Potential*. Wolters Kluwer Health, Inc. 2016.

MORE REFERENCES

- *National Center for Biotechnology Information Various Gene sites.* National Center for Biotechnology Information, U.S. National Library of Medicine. Bethesda, MD. June, 2016. Web.
- *Sleeping Beauty Awakens for Genome Engineering.* Addgene; the nonprofit plasmid repository. Cambridge, MA. June 30, 2015. Web.
- Kutlu, Elpek. et al. *Mature natural killer cells with phenotypic and functional alterations accumulate upon sustained stimulation with IL-15/IL-15Rα complexes.* Division of Cancer Immunology and AIDS, Dana Farber-Cancer Institute, Boston, MA, 02115. 14 December, 2010. Web. www.pnas.org/cgi/doi/10.1073/pnas.1012128107
- Lima, Margarida. et al. *The "ex Vivo" Patterns of CD2/C7, CD57/CD11c, CD38/CD11b, CD45RA/CD45RO, and CD11a/HLA-DR Expression Identify Acute/Early and Chronic/Late NK-Cell Activation States.* Elsevier Science (USA), 2002.
- Lopez-Vergès, Sandra. Et al. *CD57 defines a functionally distinct population of mature NK cells in the human $CD56^{dim}CD16^{bright}$ NK-cell subset.* Department of Microbiology and Immunology and the Cancer Research Institute, University of California-San Francisco, San Francisco, CA. 26 April, 2010. Web. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2981540/?report=printable
- *Gibson Assembly.* New England Biolabs Inc. Ipswich, MA. 2016. Web.14 July, 2016. https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/gibson-assembly

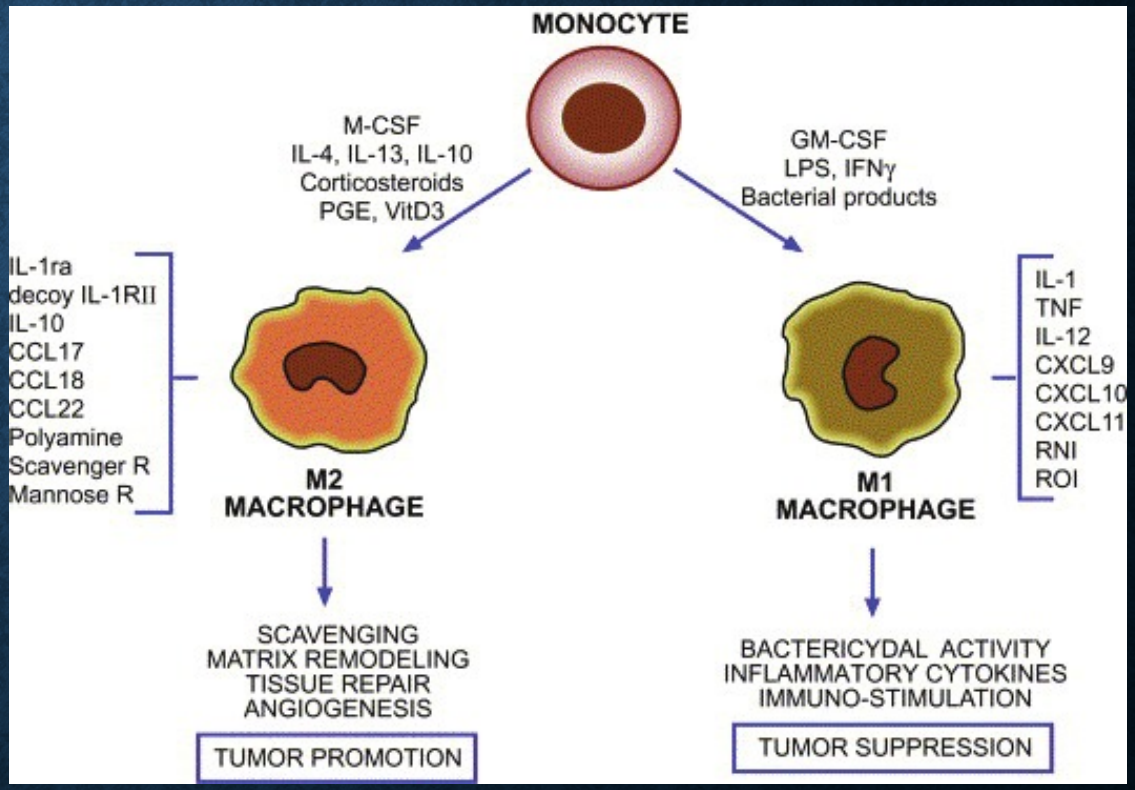
QUESTIONS

- T2A?-self cleaving peptide improving expression of more than one gene. Small peptide, ~18-22 bp,
- CD19? – Protein marker on B cells,
- MHC1? – Major histocompatibility complex I, found on all nucleated cells in the body, display peptide fragments of non-self proteins from **within** the cell to cytotoxic T-cells
- MHC2? – major histocompatibility complex II, normally found only on antigen presenting cells such as DCs, B cells, and monocytes. Present antigens derived prom **extracellular** proteins, not cytosolic proteins like MHCI.
- TGFB (Transforming growth factor beta): Superfamily of cytokines
- IL-10: anti-inflammatory cytokine
- IL-21: Cytokine that induces cell division/ proliferation in its target cells
- CD16: protein receptor that binds to IgG antibodies (through ADCC) which then activates NK cells to release cytotoxic granules containing perforin and granzyme
- ADCC (sometimes NKs need a little help finding their targets): NKs CD16 receptors recognize IgG1 and IgG2 antibodies that are made primarily by B cells and recognize bacterial, viral, and fungal pathogens. These immunoglobulins are induced by BIF (Interferon-beta 1)

Figure 1

TUMOR ASSOCIATED MACROPHAGES

- M-CSF (Macrophage Colony Stimulating Factor):
- Matrix remodeling: digestion of extracellular matrix with matrix metalloproteinases (MMPs)
- Secrete VEGF which promotes angiogenesis
- Sources of local immunosuppression that blocks the activity of anti-tumor immune cells



293T CELLS

- Human cell line derived from HEK293 cell line
- Expresses mutant version of the SV40 large T antigen which is hexamer protein capable of malignant transformation of a variety of cell types, and is involved in viral genome replication and regulation of host cell cycle.
- Used for production of retroviruses (classification of virus that can integrate its DNA into the host genome)

NATURAL KILLER (NK) CELLS

- NK cells have been shown to have anti-tumor function
- 3 ways they recognize a target
  - missing-self recognition (Ex. down-regulation of MHC-I)
  - non-self recognition (e.g. FasL, TRAIL) (ADCC)
  - stress-induced self recognition

Problem: NK response to **solid** tumors is significantly decreased

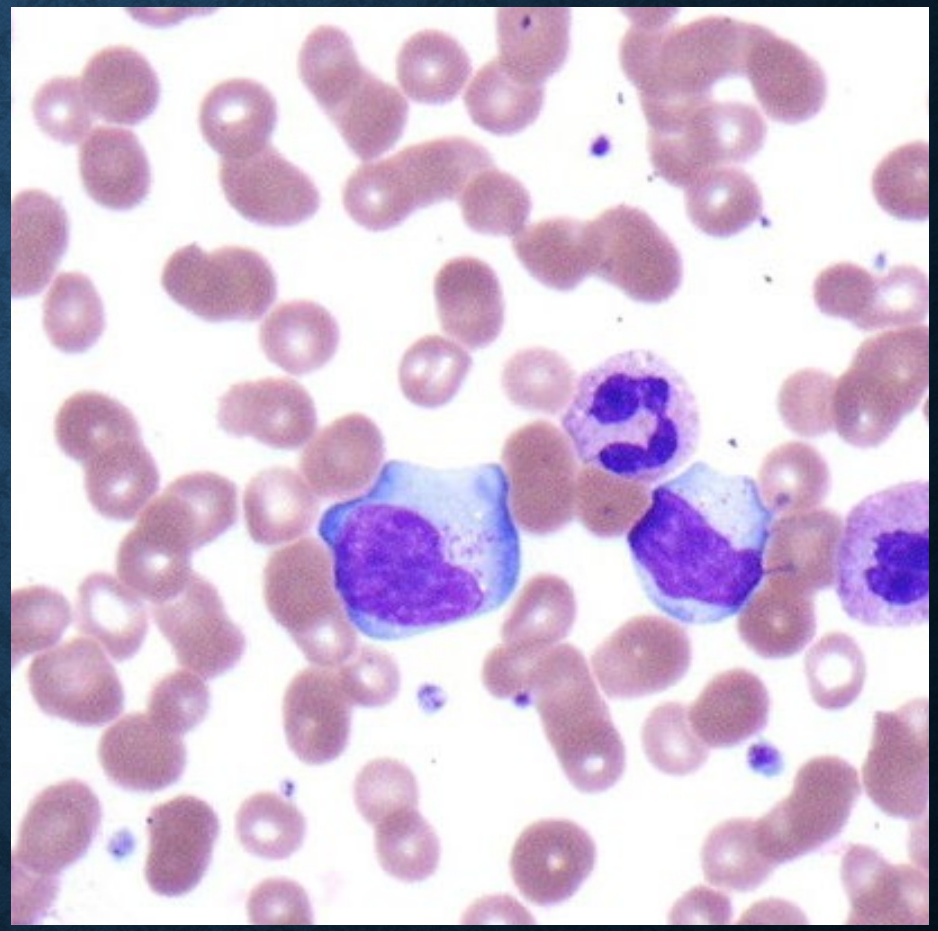


Image from American Society of Hematology Image Bank