Doctoral Dissertation Defense Announcement

Mohamed Khalil

“Defining Memory NK cell development and functions following cytomegalovirus infection”

Candidate for Doctor of Philosophy in Microbiology and Immunology
School of Graduate Studies
Medical College of Wisconsin

Committee in Charge:
Subramaniam Malarkannan, PhD (Thesis Advisor)
Scott Terhune, PhD (Thesis Advisor)
Joseph T. Barbieri, PhD
Sridhar Rao, MD PhD
Ravit Boger, MD
Colleen Lau, PhD (Cornell University)

Tuesday, May 21st, 2024
12:00 PM – 1:00 PM CST
BRI Conference Room
Zoom Meeting ID: 965 7893 5827 Passcode: vt6GAR8j
https://mcw-edu.zoom.us/j/96578935827?pwd=VWZ2NFlzZUNLM3Y2UnY5dVBTODBUdz09
**Coursework completed**

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Dissertation Summary
Defining Memory NK cell development and function following cytomegalovirus infection

Human cytomegalovirus (HCMV) is a \( \beta \)-herpesvirus that is highly prevalent in the adult population and has the ability to establish lifelong latency in healthy individuals. The innate and adaptive immune systems work closely to control viral replication, leading to a dynamic interaction between the virus and the host immune system. This interplay leads to the development of distinct immune-cell repertoires in HCMV seropositive (HCMV\(^+\)) individuals. Natural killer (NK) cells are cytotoxic lymphocytes that are required to manage and control HCMV infections and possess strong anti-tumor activity. NK cells utilize their activating NKG2C/CD94 receptor complex to mount a response to HCMV-infected cells that present HLA-E molecules loaded with the HCMV UL40-derived peptide. As a result, HCMV\(^+\) individuals possess higher levels of NKG2C\(^+\) NK cells that contract to form a subset of memory-like cells. These NKG2C\(^+\) memory NK cells have enhanced anti-tumor and cytotoxic characteristics and therefore can be utilized in a variety of anti-tumor cellular therapies. However, the development and the underlying transcriptional mechanisms maintaining and sustaining these memory cells are not well understood. Given their potent cancer immunotherapeutic potential, my PhD thesis primarily aims to transcriptionally define this memory NK cell subset and explore their developmental origins.

Recent work by our lab and others have demonstrated that HCMV\(^+\) individuals exhibit significantly higher proportions of NKG2C\(^+\) memory NK cells in their peripheral blood. We found NKG2C\(^+\) memory NK cells have elevated transcriptional levels of \( CD3 \), \( CD52 \) and \( KLRC2 \). To expand on these findings, we isolated NK cells from the human spleen, a secondary lymphoid organ crucial for initiating memory immune responses. To investigate if the human spleen is serving as one of the sites involved in housing and sustaining NKG2C\(^+\) memory NK cells, we obtained eight healthy adult human spleens from four HCMV seronegative (HCMV\(^-\)) and four HCMV\(^+\) donors. Donor median age was 52 [IQR 47.5-56.5], 50\% (n=4) were identified as female and 50\% (n=4) were identified as male, and 50\% (n=2) of females were HCMV\(^-\) and 50\% (n=2) of females were HCMV\(^+\). The human spleens were provided by the Versiti Organ Donor Center of Wisconsin and were processed to a single cell suspension. In line with previous findings in the peripheral blood of HCMV\(^+\) individuals, we observed significantly elevated proportions of NKG2C\(^+\) NK cells in the spleens of HCMV\(^+\) donors. This observed significance of higher NKG2C\(^+\) NK cells was consistent across all HCMV\(^+\) donors when compared to our HCMV\(^-\) donors.

To investigate the molecular mechanisms involved in the maintenance and persistence of memory NKG2C\(^+\) NK cells, we performed single-cell RNA sequencing (scRNA-seq), using the sorted NK cells from all eight donors. Using unbiased clustering analysis, we identified and characterized four distinct NKG2C\(^+\) splenic NK cell subsets. Our findings indicated that the relative composition of these NKG2C\(^+\) subsets was highly influenced by the HCMV serostatus of the donors. Specifically, we observed that HCMV\(^+\) donors had significantly higher levels of NKG2C\(^\text{Hi}\) memory NK cells when compared to the HCMV\(^-\) donors. Our findings indicated that the relative composition of these NKG2C\(^+\) subsets was highly influenced by the HCMV status of the donors. Specifically, we observed that HCMV\(^+\) donors had significantly higher levels of NKG2C\(^\text{Hi}\) memory NK cells when compared to the HCMV\(^-\) donors. This NKG2C\(^\text{Hi}\) memory NK subset had significantly higher expression of \( NKG2C \), \( CD52 \), \( CD3\varepsilon \), and \( IL7R \) and significantly lower expression of \( FCER1G \), \( KLRC1 \), \( CD247 \), \( ZBTB16 \), \( SYK \), and \( SH2D1B \). These findings suggest NKG2C\(^\text{Hi}\) memory NK cells possess unique transcriptional and molecular mechanisms that may contribute to their ability to persist over time.

To explore the developmental cell fate of NKG2C\(^\text{Hi}\) memory NK, we utilized computational modeling from both the Monocle 2 and Monocle 3 software’s to track how NKG2C\(^+\) cells transition between transcriptomic states. Monocle 3 analysis yielded a simple early-to-late cell fate trajectory that was projected onto our UMAP plot. We found that NKG2A\(^\text{Hi}\) NKG2C\(^+\) subset served as the early timepoint.
in the developmental trajectory, ultimately leading to the development of NKG2C<sup>Hi</sup> memory NK. The Monocle 2 analysis produced unique developmental trajectory plots, allowing us to visualize distinct developmental pathways and transitions. Interestingly, we observed a unique branch-point exclusive to the HCMV<sup>+</sup> donors that expressed significantly higher transcriptional levels of CD3<sup>e</sup> and IL7R. These data collectively demonstrate that HCMV exposure impacts the developmental trajectories of NK cells and influence the developmental origins of NKG2C<sup>Hi</sup> memory NK cells.

Together, the work presented in this dissertation demonstrates that HCMV infection can promote the formation of NKG2C<sup>Hi</sup> memory NK cells in the human spleen that displays a unique transcriptional and developmental profile. These findings may ultimately prove useful in the future isolation and application of memory NK cells in cellular immunotherapies.
Mohamed Khalil  
mokhalil@mcw.edu

**Education**
August 2018 – present  
MD/PhD Candidate (anticipated May 2026)  
Medical College of Wisconsin, Milwaukee, WI

August 2014 – May 2016  
B.S. Molecular and Cellular Biology  
Illinois State University, Normal, IL

January 2012 – May 2014  
A.A.S Applied Sciences  
Harry S. Truman College Chicago, IL

**Research Experience**
August 2020 – present  
Antiviral functions of NK cells, characterizing memory NK cells following HCMV infection, congenital CMV  
MCW/BRI (Malarkannan & Terhune labs)

August 2018 – March 2020  
Short-term morbidity and mortality outcomes in CRS/HIPEC in patients with appendiceal cancers  
MCW Department of Surgery (Dr. Harveshp Mogal)

January 2015 – May 2016  
Utilizing the nematode *C. elegans* as a model to study the molecular and neurological basis of Angelman Syndrome  
Illinois State University (Dr. Andres Vidal-Gadea)

October 2014 – January 2015  
Molecular machinery involved in *C. elegans* magnetotaxis behavior and function  
Illinois State University (Dr. Andres Vidal-Gadea)

**Grant Funding**
July 2022 – present  
TL1-TR001437  
“Defining the immunological consequences of congenital cytomegalovirus infection”

**Publications**


3. Khalil M, Terhune SS, Malarkannan S. Single cell transcriptomes from HCMV\(^+\) donors reveal a unique NKG2C\(^{Hi}\) subset that represents true memory NK cells. *Journal of Immunology* 2023;244.01 doi: 10.104049/jimmmunol.210.Supp.244.01.


**Abstracts**

**Oral presentations**

1. **Khalil M**, Terhune SS, Malarkannan S. Single cell transcriptomes from fetuses exposed to HCMV during gestation reveals a distinct subset of memory-like NKG2C+ fetal NK cells. American Association of Immunologist 2024. Chicago, IL. May 2024

2. **Khalil M**, Terhune SS, Malarkannan S. Characterizing the single-cell transcriptomes of fetal NK cells isolated from the umbilical cord of fetuses exposed to human cytomegalovirus during gestation. Association for Clinical and Translation Sciences. Las Vegas, NV. April 2024

3. **Khalil M**, Terhune SS, Malarkannan S. Memory NK cells are regulated by a unique transcriptional program allowing for their persistence in HCMV+ individuals. Autumn Immunology Conference. Chicago, IL. November 2023

4. **Khalil M**, Terhune SS, Malarkannan S. Cytomegalovirus infection induces the generation of NKG2CHi fetal NK cells in the umbilical cord. Midwest TL1 Summit. Wauwatosa, WI. September 2023

5. **Khalil M**, Terhune SS, Malarkannan S. Interleukin-7 Receptor (IL-7R) and CD3e are necessary components for the development and function of NKG2CHi adaptive NK cells during HCMV infections. 13th Annual Immunology Scientific Retreat, Center for Immunology. Wauwatosa, WI. June 2023


7. **Khalil M**, Terhune SS, Malarkannan S. Single-cell transcriptome of human adaptive NK cells reveal a unique transcriptional program that governs its development and function. Autumn Immunology Conference. Chicago, IL. November 2022


Poster presentations

2. Khalil M, Terhune SS, Malarkannan S. The single-cell transcriptomes of fetal NK cells unveil a distinctive subset crucial for the management of congenital cytomegalovirus infection. All Things Data Science Symposium. Milwaukee, WI. April 2024

3. Khalil M, Terhune SS, Malarkannan S. Characterizing the single-cell transcriptomes of fetal natural killer cells isolated from the umbilical cord of fetuses exposed to human cytomegalovirus during gestation. Association for Clinical and Translation Sciences. Las Vegas, NV. April 2024

4. Khalil M, Terhune SS, Malarkannan S. Single-cell transcriptomes of fetal NK cells reveal a unique NKG2C^{Hi} population that helps fetus manage congenital cytomegalovirus infection. MCW Research Week. Wauwatosa, WI. March 2024

5. Khalil M, Terhune SS, Malarkannan S. Transcriptomic profiling of fetal NK cells derived from the umbilical cord of fetuses exposed to human cytomegalovirus during gestation. Department of Medicine. Wauwatosa, WI. March 2024


7. Khalil M, Terhune SS, Malarkannan S. Human cytomegalovirus infection promotes the formation of NKG2C^{Hi} memory NK cells that express elevated levels of IL-7R and cyto-CD3ε. American Society of Hematology. San Diego, CA. December 2023

8. Khalil M, Terhune SS, Malarkannan S. Single-cell transcriptome of HCMV seropositive fetuses reveals a unique subset of NKG2C^{+} cells that represent adaptive NK cells. 33rd Annual Graduate School Poster Session. Milwaukee, WI. December 2023

9. Ishaq A, Khalil M, Terhune SS, Malarkannan S. Single-cell transcriptomes of NK cells responding to MCMV infection reveal a distinct Ly49H^{Hi} memory subset emerging 7 days post-infection. Autumn Immunology Conference. Chicago, IL. November 2023

10. Khalil M, Terhune SS, Malarkannan S. Memory NK cells are regulated by a unique transcriptional program allowing for their persistence in HCMV+ individuals. Autumn Immunology Conference. Chicago, IL. November 2023


12. Khalil M, Terhune SS, Malarkannan S. Cytomegalovirus infection induces the generation of NKG2C^{Hi} fetal NK cells in the umbilical cord. Midwest TL1 Summit. Wauwatosa, WI. September 2023

13. Khalil M, Terhune SS, Malarkannan S. Infection by human cytomegalovirus (HCMV) stimulates the development of memory NK cells characterized by increased expression of cyto-CD3ε. 38th Annual MD-PhD National Student Conference. Copper Mountain, CO. July 2023

14. Khalil M, Terhune SS, Malarkannan S. Interleukin-7 Receptor (IL7R) and CD3ε are necessary components for the development and function of NKG2C^{Hi} adaptive NK cells during HCMV
infections. 13th Annual Immunology Scientific Retreat, Center for Immunology. Wauwatosa, WI. June 2023

15. Khalil M, Terhune SS, Malarkannan S. Single cell transcriptomes from HCMV+ donors reveal a unique NKG2C\textsuperscript{Hi} subset that represents true memory NK cells. American Association of Immunologist 2023. Washington, D.C. May 2023


17. Khalil M, Terhune SS, Malarkannan S. Interleukin-7 Receptor (IL7R) and CD3\textgreek{e} are necessary for the development and function of NKG2C+ memory-like NK cells during HCMV infections. 6th Annual Graduate School Symposium. Milwaukee, WI. April 2023

18. Khalil M, Terhune SS, Malarkannan S. Single-cell transcriptomes reveal a unique NKG2C\textsuperscript{Hi} population representing memory NK cells. MCW Research Week. Wauwatosa, WI. March 2023

19. Khalil M, Terhune SS, Malarkannan S. Single-cell transcriptome from the spleen of HCMV+ donors reveal a unique subset of NKG2C+ population that represent true memory NK cells. Department of Medicine. Wauwatosa, WI. March 2023


21. Khalil M, Terhune SS, Malarkannan S. Single-cell transcriptome of HCMV seropositive individuals reveals a unique subset of NKG2C+ cells that represent adaptive NK cells. 32nd Annual Graduate School Poster Session. Wauwatosa, WI. October 2022

22. Khalil M, Terhune SS, Malarkannan S. Transcriptome of human adaptive NK cells reveal a unique transcriptional program that governs its development and function. 11th Annual CTSI Milwaukee Regional Research Forum. Milwaukee, WI. October 2022

23. Khalil M, Terhune SS, Malarkannan S. Transcriptomic profiling of human Adaptive NK cells derived from HCMV seropositive individuals. 1st Annual Midwest TL1 Research Summit. Madison, WI. September 2022

24. Khalil M, Terhune SS, Malarkannan S. Defining the Human Cytomegalovirus manipulation of NK cells at a single-cell resolution. 12th Annual Immunology Scientific Retreat, Center for Immunology. Wauwatosa, WI. June 2022


27. Khalil M, Vidal-Gadea AG. Loss of function in the nematode UBE3A ortholog leads to neurological impairments in a C. elegans model of Angelman Syndrome. Animal Behavior Conference at Indiana University, Bloomington, IN. April 2016 [Undergraduate Presentation Award]


**Journal Reviewer**
1. Journal of Experimental Medicine
2. Frontiers in Immunology
3. iScience
4. Scientific Reports

**Awards and Honors**

- **May 2024**: Top Abstract, American Association of Immunologist
- **April 2024**: Outstanding Abstract, Association for Clinical and Translational Science
- **January 2024**: Heath Foundation Travel Award, Department of Medicine MCW
- **November 2023**: John Wallace Diversity Award, Autumn Immunology Conference
- **October 2023**: Center for Immunology Travel Award, C4I MCW
- **September 2023**: Graduate School 2023-2024 Diversity Award, MCW
- **July 2023**: Diversity Travel Award, National MD-PhD Student Conference
- **June 2023**: Top Abstract Award, Annual Immunology Scientific Retreat MCW
- **May 2023**: Trainee Abstract Award, American Association of Immunologist
- **April 2023**: Outstanding Trainee Award, Association for Clinical and Translational Science
- **March 2023**: Best Poster Presentation, MCW Department of Medicine
- **December 2021**: Graduate Student Travel Award, MCW
- **June 2020**: Medical Student Summer Research Program, MCW
- **February 2020**: Outstanding Medical Student Presentation Award, Society of Surgical Oncology International Conference
- **August 2018 – 2020**: Medical Student Presidential Scholar Award, MCW
- **May 2016**: Outstanding Undergraduate Oral Presentation, Phi Sigma Symposium
- **March 2016**: Undergraduate Oral Presentation Award, Animal Behavior Conference

**Invited Talks**

- **January 2024**: “Fetal NK cells: Unraveling Their Role During Congenital Cytomegalovirus Infection” Illinois State University Molecular Immunology Seminar
- **September 2023**: “Human cytomegalovirus infection drives the formation of NKG2C^Hi memory NK cells that express elevated levels of IL-7R and cyto-CD3e” Component & Module Leaders CTSI
- **August 2022**: “Immunological Consequences of Congenital Cytomegalovirus Infection” TL1 Predoctoral Training Grant NIH Renewal Review Board
- **September 2021**: “Single cell transcriptomes of Natural Killer Cells during viral infections” Illinois State University School of Biological Sciences Seminar

**Leadership Positions**

- **February 2024 – present**: Student Ambassador - MCW Social Media Team
- **December 2023 – present**: Admissions Committee Student Chair - Medical College of Wisconsin Medical Scientist Training Program
- **November 2023 – present**: MSTP Student Council G4/5 Representative - Medical College of Wisconsin Medical Scientist Training Program
- **January 2023 – present**: Mentorship Chair - Mentoring and Advocating for Diverse Physician Scientist (MAPS)
- **August 2023 – present**: Founder & President - Muslim Professionals Mentoring Program
- **September 2020 – present**: Medical Student Interviewer - Medical College of Wisconsin Medical School Admissions Committee
- **September 2020 – present**: MD-PhD Student Interviewer - Medical College of Wisconsin Medical Scientist Training Program
### Professional Development

**Teaching and tutoring**
- **July 2022 – present**
  - **Tutor** - SEA literacy Milwaukee
- **August 2021 – August 2022**
  - **Small Group Facilitator** - M1 physiology Case Based Discussions, Medical College of Wisconsin
- **August 2020 – May 2022**
  - **Tutor** - USMLE-STEP1, MedSchoolCoach
- **August 2020 – May 2021**
  - **Small Group Instructor** - Academic Enhancement USMLE-STEP1 Prep, Medical College of Wisconsin
- **October 2018 – March 2020**
  - **Cadaver Educator** - Cadavers as Educators, Medical College of Wisconsin

**Conference judge**
- **March 2024**
  - **Poster Judge** - Department of Medicine Research Retreat
- **April 2023**
  - **Poster Judge** - Association of Clinical and Translational Sciences
- **November 2022**
  - **Poster Judge** - Autumn Immunology Conference

**Clinical engagement**
- **September 2018 – present**
  - **Volunteer** - Saturday Clinic for the Uninsured, Medical College of Wisconsin
- **July 2020 – July 2023**
  - **Clinical Continuity Track** - Saturday Clinic for the Uninsured, Medical College of Wisconsin
- **October 2018 – March 2020**
  - **Phlebotomist** - Saturday Clinic for the Uninsured, Medical College of Wisconsin
- **August 2018**
  - **Adult & Pediatric CPR/AED & First Aid** - American Red Cross

**Mentorship**
- **June 2023 – present**
  - Mentoring and Advocating for Diverse Physician Scientist, Medical College of Wisconsin
- **July 2022 – present**
  - SEA Literacy Milwaukee
- **October 2018 – present**
  - Pre-med Pair Up (PMPU), Medical College of Wisconsin
August 2018 – May 2023  Pre-health Student Organization, Illinois State University School of Biological Sciences
May 2022 – August 2022  Summer Program for Undergraduate Research (SPUR), Medical College of Wisconsin

**Students Mentored**

October 2023 – present  Ahmad Farooq, Loyola University Chicago, pre-med undergraduate student
July 2023 – present  Ali Ishaq, University of Wisconsin-Milwaukee, undergraduate student in the Malarkannan lab
June 2023 – present  Manaar Jan, University School of Milwaukee, highschool student in the Malarkannan lab
December 2022 – present  Tariq Abdelhamid, Illinois State University, pre-med undergraduate student
September 2022 – present  Mohammed Hommedia, Medical College of Wisconsin, *current* medical student
June 2023 – August 2023  Manaar Jan, University School of Milwaukee, highschool student in the Malarkannan lab
December 2021 – July 2022  Abraham Valazquez, Rush Medical College, *current* medical student
June 2021 – August 2021  Blessed Ikuobolati, Summer Program for Undergraduate Research, SPUR student
July 2020 – July 2022  Belal Abuzir, Rosalind Franklin University of Medicine and Science, podiatry student
June 2018 – May 2020  Andrew Schuler, University of Chicago Medical School, medical student

**Community Outreach**

July 2022 – present  Science Tutor- SEA Literacy Milwaukee
June 2021 – present  Tour Guide- Alumni Center, MCW
January 2019 – present  Veteran Interviewer- My life My story for Veterans, MCW
September 2018 – present  Annual Science Fair Judge- Heritage Christian Private School
October 2018 – July 2022  Youth Volunteer Coordinator- Islamic Society of Milwaukee
August 2018 – August 2020  Volunteer- Student Health Initiative Pipeline Program, MCW
September 2016 – July 2018  Tutor- YWCA Smart Sprouts

**Service Trips**

May 2018  Volunteer- Zaatari Refugee Camp, Mafraq, Jordan
December 2017  Clinical Volunteer- Medlife Movement, Lima, Peru
March 2017  Site Leader- Our House Shelter, Little Rock, AR
April 2016  Service Leader- Habitat for Humanity, Winder, GA
November 2015  Volunteer- Camp Cedar Lodge, Lawrence, MI

**Memberships and Professional Societies**

American Association of Immunologist (AAI)
American Society of Hematology (ASH)
Association of Clinical and Translational Science (ACTS)
American Physician Scientist Association (APSA)
Wisconsin Medical Society (WMS)
American Medical Association (AMA)
American College of Physicians (ACP)
American College of Surgeons (ACS)