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TITLE: Enhancing Natural Killer Cell Mediated Targeting and Responses to Myeloid Leukemias

PRINCIPAL INVESTIGATOR: Martin Felices

CONTRACTING ORGANIZATION: Regents of the University of Minnesota
Minneapolis, MN 55455

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Martin Felices

E-Mail: mfelices@umn.edu

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Regents of the University of
Minnesota
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200 Oak Street SE
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Myeloid Leukemias represent a growing problem in our aging population. Although treatment of these leukemias has advanced considerably over the past couple of decades, most of these myeloid leukemias still have poor prognosis, particularly in the elderly, and require hematopoietic cell transplants to fully kill the tumor, which is both costly and risky. A great deal of excitement has recently been placed on cellular therapies to treat tumors. Natural Killer (NK) cell immunotherapies could be used to treat myeloid tumors, but these cells require help in being targeted to the tumor and overcoming inhibitory signals that help the tumor escape recognition and killing. Our group has recently described small bivalent molecules (termed BiKEs) that target NK cells to myeloid tumors and induce NK cell mediated tumor killing. Though these molecules show promise, the work scope proposed here builds on them through incorporation of signals that will maximize NK cell function. This will be achieved by creating trivalent molecules (termed TriKEs) that target and activate NK cells but also either block inhibitory signals provided by the tumor cells, further enhance the activating signals provided by the BiKE, or drive expansion and maintenance of the NK cells killing the tumor cells.

15. SUBJECT TERMS
NK – Natural Killer, CML – Chronic Myeloid Leukemia, MDS – Myelodysplastic Syndromes, AML – Acute Myeloid Leukemia, BiKE – Bi-specific Killer Engager, TriKE – Tri-specific Killer E

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1. Intro (Year-2):

Myeloid leukemias represent a growing problem in our aging population. In military personnel the incidence of myeloid malignancies is increased due to exposure to ionizing radiation, chemicals, and other agents during deployment. Although treatment of these leukemias has advanced considerably over the past couple of decades, most of these myeloid leukemias still have poor prognosis, particularly in the elderly, and require hematopoietic stem cell transplants to fully kill the tumor. These transplants are costly, risky, and quite harsh on the patient, especially if the patient is older and frail. A great deal of excitement has recently been placed on cellular therapies to treat tumors. Rather than eradicating the tumors through chemicals and radiation, cellular therapies enhance immune function in the patients so the immune cells themselves can kill the tumors. One type of immune cells whose role is to find and kill tumors is the Natural Killer (NK) cell. Upon clinical diagnosis of myeloid leukemia the NK cells require help in being targeted to the tumor and overcoming inhibitory signals that help the tumor escape recognition and killing. Our group has described small bivalent molecules, termed BiKEs (Bi-specific Killer Engagers), which target NK cells to myeloid tumors and induce NK cell mediated tumor killing. The targeting in the BiKE is mediated by an anti-CD33 scFv, as CD33 is expressed in a number of these myeloid tumors, while the NK cell activation is mediated by an anti-CD16 scFv, as CD16 is a potent NK cell activating receptor. Recently we have published on a TriKE (Tri-specific Killer Engager) molecule that incorporates the cytokine IL-15 into the BiKE platform to enhance NK cell function, demonstrating our capability to expand on this platform. Though these molecules show promise, the work scope proposed here builds on them through incorporation of other signals that will maximize NK cell function. Our research will first optimize the platform to better induce killing of myeloid malignancies. This will be achieved in several ways: 1) strengthening the main activating signal through incorporation of a strong single domain anti-CD16 arm into the platform in the place of the previous CD16 scFv; 2) swapping the main CD16 activating signal for a more NK cell specific NKG2C activating signal; 3) swapping the myeloid tumor targeting domain, targeting CLEC12A instead of CD33, in order to reduce toxicities and enhance myeloid cancer stem cell control; 4) incorporating different activating signals (IL-21, IL-12 or 41BB-L) in the place of the IL-15; or 5) blocking inhibitory signals (PD-L1) provided by the tumor cells that interact with the PD-1 on NK cells. We believe these next generation TriKE molecules will further induce tumor killing and drive expansion and maintenance of the NK cells in order to maximize NK cell based immunotherapies. Attaining an immunotherapy that would bypass the need for costly and dangerous hematopoietic stem cell transplants but also diminish the rate of relapse in myeloid malignancies would greatly impact the way we treat these patients. Of note, the potential of these TriKE molecules is highlighted by the fact that our earlier version of the TriKE molecule incorporating IL-15 is headed into a Phase I clinical trial at the University of Minnesota late 2018 for treatment of refractory AML and high risk MDS.

2. Key words (Year-2):

NK – Natural Killer
CML – Chronic Myeloid Leukemia
MDS – Myelodysplastic Syndromes
AML – Acute Myeloid Leukemia
TriKE – Tri-specific Killer Engager
scFv – Single-chain variable fragment
ADCC – Antibody-Dependent Cell-mediated Cytotoxicity
IL- – Interleukin
KIR – Killer-cell Immunoglobulin-like Receptor
161533 – anti-CD16 x IL-15 x anti-CD33 TriKE
1633KIR – anti-CD16 x anti-CD33 x anti-KIR TriKE
1633NKG2A – anti-CD16 x anti-CD33 x anti-NKG2A TriKE
1633PDL1 – anti-CD16 x anti-CD33 x anti-PD-L1 TriKE
161233 – anti-CD16 x IL-12 x anti-CD33 TriKE
162133 – anti-CD16 x IL-21 x anti-CD33 TriKE
1633137 – anti-CD16 x anti-CD33 x anti-CD137 TriKE
NKG2C – Natural Killer Group 2 C
CLEC12A - C-Type Lectin Domain Family 12 Member A
cam16 – camelid anti-CD16 single domain antibody
V_HH – Heavy domain antibody (also called single domain antibody or nanobody)

3. Accomplishments (Year-2):

The major goal of this proposal is to generate novel Tri-specific Killer Engager (TriKE) molecules in order to improve natural killer (NK) cell based immunotherapies against myeloid malignancies including AML, MDS, and CML. The molecules proposed build on a Bi-specific Killer Engager (BiKE) platform containing an anti-CD33 scFv, for myeloid tumor targeting, joined by a linker to an anti-CD16 (termed 1633 henceforth), to robustly induce NK cell activation. Six new TriKEs were proposed in this grant to enhance the NK cell activity mediated by the former 1633 BiKE; three targeting blockade of inhibitory pathways on NK cells (1633KIR, 1633NKG2A and 1633PDL1) and three enhancing NK cell activation (161233, 162133 and 1633137). To achieve this goal the proposal was split up into three major tasks, each occupying roughly one year of the proposal. The first major task, which takes place in year 1, involves construction of the TriKEs, as well as obtaining local IRB and DoD HRPO approval. The second major task, in year 2, involves testing of the three TriKEs targeting inhibitory pathways on NK cells. The third and last major task, in year 3, involves testing of the three TriKEs targeting enhancement of NK cell function via cytokines and co-stimulatory receptors.

In the course of incorporating the scFvs the inhibitory scFvs and testing them (outlined in major tasks for year 1 and year 2) we have discovered that two of the inhibitory TriKEs proposed are not functional. Despite testing multiple scFvs arms for NKG2A, we haven't been able to properly express an NKG2A TriKE. Our next optimization step for this TriKE will be to try to express it in a bacterial system instead of our mammalian system since we have had success with that system in the past. The KIR TriKE has also proven problematic, in particular demonstrating reduced binding of the KIR scFv arm to KIR once incorporated into the TriKE backbone. Due to this no enhancement of function has been seen with this TriKE thus far. We are currently testing a number of linker variations and swapping out the anti-CD16 scFv domain to the cam16 (V_HH domain, discussed later on) to try to optimize this TriKE as well. The PD-L1 TriKE was originally but we believe that swapping in the cam16 domain has solved that problem based on recent data generated on a cam16PD-L1 BiKE showing enhanced inflammatory functionality against PD-L1 expressing line THP-1 (**Figure 1)**. THP-1 cells express PD-L1, which restricts NK cell functionality, but they upregulate PD-L1 even more after overnight incubation with IFNγ, which the tumor cell uses as a defense mechanism against inflammatory immune cells (like NK cells) which secrete IFNγ. When we incubate NK cells with THP-1 cells and cam16PDL1 BiKE we get about 3.5-fold more IFNγ production by NK cells than you do when you treat with no TriKE. The increase in inflammatory cytokine secretion is even greater (8-fold) when THP-1 cells have higher PD-L1 expression due to overnight incubation with IFNγ, indicating that besides blocking the inhibitory PD-1 (on NK) and PD-L1 (on tumor) interaction, this molecule might also drive targeted activation against PD-L1 expressing cells. These results are very encouraging and we are creating a cam16xPD-L1xCD33 (cam16PDL133) TriKE to enhance the targeting of myeloid malignancies, which we will test as planned before.

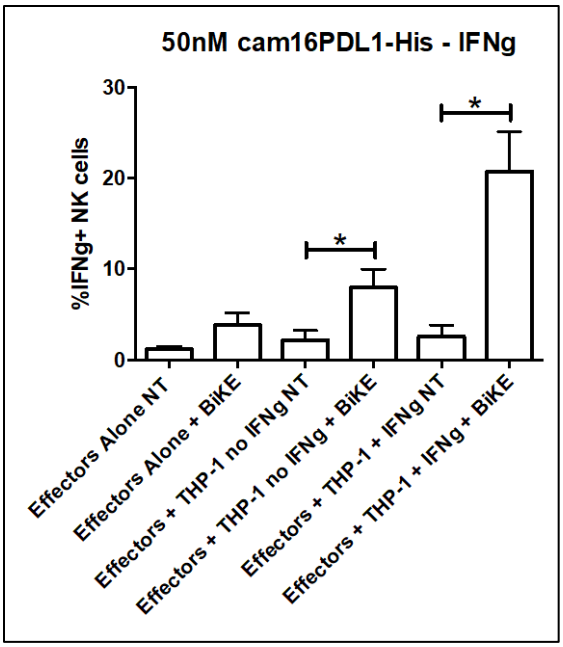

Figure 1: cam16PDL1 BiKE induces enhanced inflammatory function against myeloid tumor targets

As discussed in the previous progress report, and alluded above, the original TriKE platform consisted of an anti-CD16 scFv arm, an IL-15 arm, and an anti-CD33 arm (termed 161533 TriKE) but demonstrated reduced functionality and limited the number of other scFvs present in the molecule. We have optimized this by swapping in a humanized anti-CD16 arm derived from a llama single domain antibody (V_HH), which we call cam16, in the place of the anti-CD16 scFv. Due to some of the limitations with the inhibitory TriKEs, described in the previous paragraph, over the past year we have shifted our focus and dedicated a good amount of time to characterizing and testing this cam16xIL-15xCD33 (cam161533 TriKE). Across the board, the cam161533

TriKE induces more NK cell activation, measured by surface CD69 surface expression (**Figure 2A**) and degranulation as measure by CD107a (**Figure 2B)** on NK cells, more HL-60 myeloid tumor killing (**Figure 2C**), and more NK cell proliferation (**Figure 2D**). Taken together we believe this second generation cam161533 TriKE molecule is far superior to the first generation 161533 TriKE molecule, which is about to enter the clinic. We will submit a manuscript for this project by the end of the year and currently in the process of generating a GMP batch of this drug as a follow up clinical trial to the first generation TriKE.

As an alternative to the NKG2A TriKE, we decided to pursue a TriKE against NKG2C, an activating receptor within the NKG2A family. NKG2C is expressed in a population of more differentiated NK cells that arises in people that have been previously exposed to CMV. The interesting thing about NKG2C is that we can use it as an alternative to targeting activation of NK cells through CD16. Unlike CD16, NKG2C is not clipped upon activation and is not expressed on neutrophils and macrophages, making it a much more specific NK cell targeted agent. We generated an anti-NKG2C(scFv)xIL-15xanti-CD33(scFv) targeted TriKE and tested it in vitro on NKG2C low and NKG2C high NK cells. Results indicate that the TriKE selectively induces NK cell degranulation on the NK cells that express

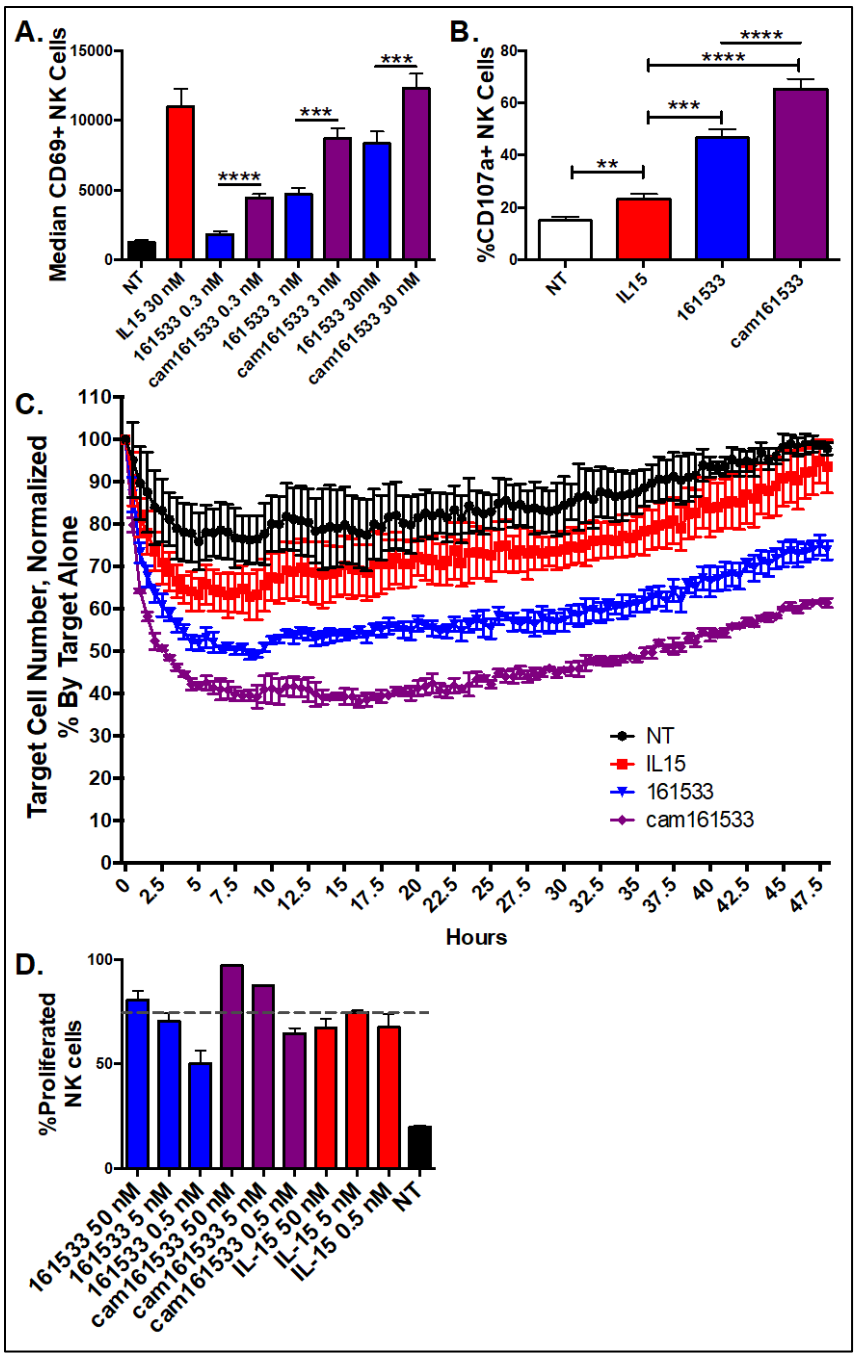

Figure 2: cam161533 TriKE is more functional than 161533 TriKE

NKG2C (High NKG2C+) when incubated with myeloid tumor targets, THP-1s, but does not induce degranulation of NK cells that don't express NKG2C (Low NKG2C+) when tested with THP-1 cells (**Figure 3A**). In a CellTrace dye dilution proliferation assay the NK2C TriKE selectively delivers proliferation signal to NKG2C expressing cells vs. IL-15, which induces proliferation on all NK cells (**Figure 3B, bottom right vs. top right**). Finally, using induced pluripotent stem cells we generated a population of NK cells that uniformly expressed NKG2C or NKG2C and DAP12, an adaptor of NKG2C meant to enhance function. We tested the NKG2C TriKE in this population and show increased dynamic killing of tumor cells versus not TriKE treatment (**Figure 3C, dark blue and red vs. light blue and red**). Taken together this data shows that we have created a functional NKG2C activating TriKE that can deliver specific activating signals to NK cells. We are finishing up experiments on this project and hope to submit a manuscript early to mid next year.

The issues with the inhibitory TriKEs have also allowed us to push up the research on activating TriKEs. Besides progress described in the previous progress report on the IL-21 activating TriKE, we have worked on

the IL-12 activating TriKE on the past year. This TriKE was initially geared to improve the inflammatory component of the NK cell response and it does just that, as shown on **Figure 4A**. However, using an assay in which NK cells are forced to exhaustion due to repeated exposure to tumor cells, we have shown that the IL-12 TriKE can also rescue NK cells from exhaustion (**Figure 4B**). We believe that NK cell exhaustion might very well be one of the major barriers to effective NK cell immunotherapy against myeloid malignancies, so these findings bare a lot of relevance. We are hoping to submit a manuscript on this project by mid-late next year.

Finally, in terms of projects of projects generated as an alternative to the inhibitory TriKEs not currently working, we have been exploring targeting of other myeloid tumor antigens. While CD33, the myeloid tumor antigen proposed originally, is broadly expressed on AML, MDS and CLL, it is also broadly expressed in normal myeloid cells, albeit at a lower level, raising concerns of on target/off tumor toxicities. CLEC12A, also known as CLL1, is an interesting alternative CD33 as it is not expressed normally on myeloid cells but is expressed in AML, MDS as well as myeloid tumor cancer stem cells. Also, 70% of CD33^{-} AMLs express CLEC12A, making it a valuable target for CD33 antigenic escape strategies. We have generated a cam16xIL15xanti-CLE12A TriKE and tested it in a number of settings. This molecule induces NK cell proliferation (**Figure 5A**), NK cell degranulation against myeloid tumor cell lines (**Figure 5B**), and can induce specific primary patient MDS cell killing in an in vitro assay (**Figure 5C**). We are extremely excited about the potential of targeting CLEC12A as an alternative to CD33 and are planning to submit this manuscript early next year. Though our proposed studies have changed slightly due to issues with some of the inhibitory TriKEs, we have been extremely productive with alternative projects (cam161533 TriKE, NKG2C1533 TriKE, and cam1615CLEC12A TriKE) and have pulled up ahead with the IL-12 TriKE, proposed for year 3.

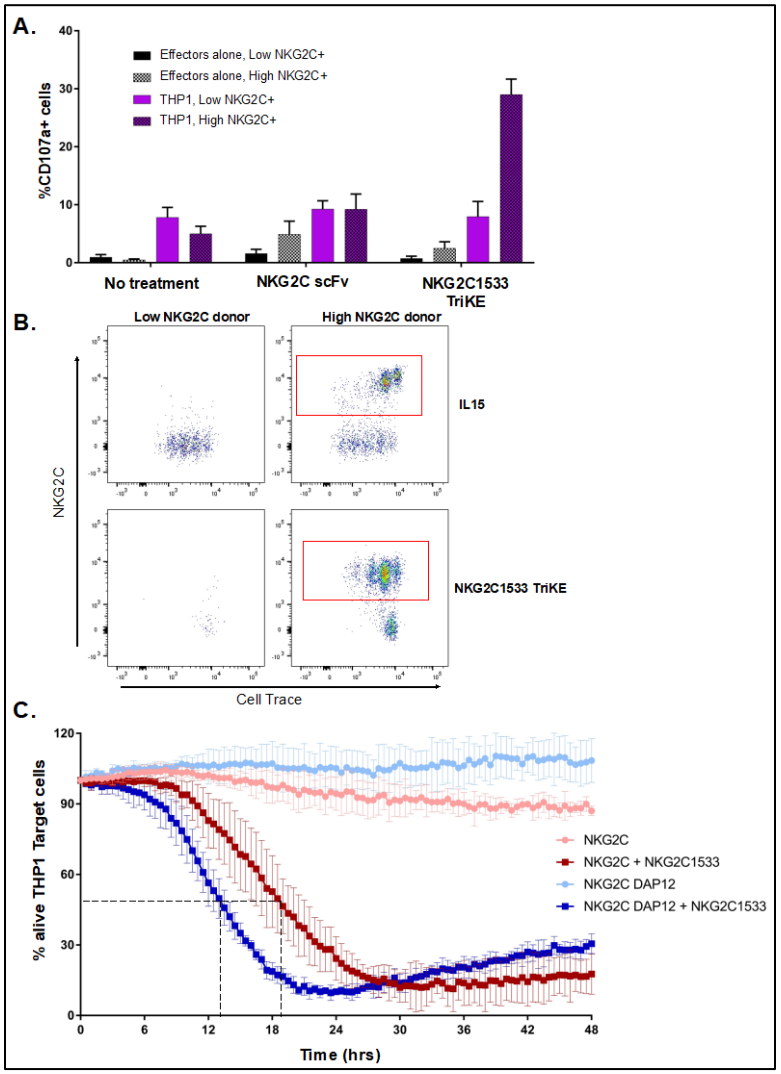

Figure 3: NKG2C TriKE selectively targets and activates NKG2C+ NK cells

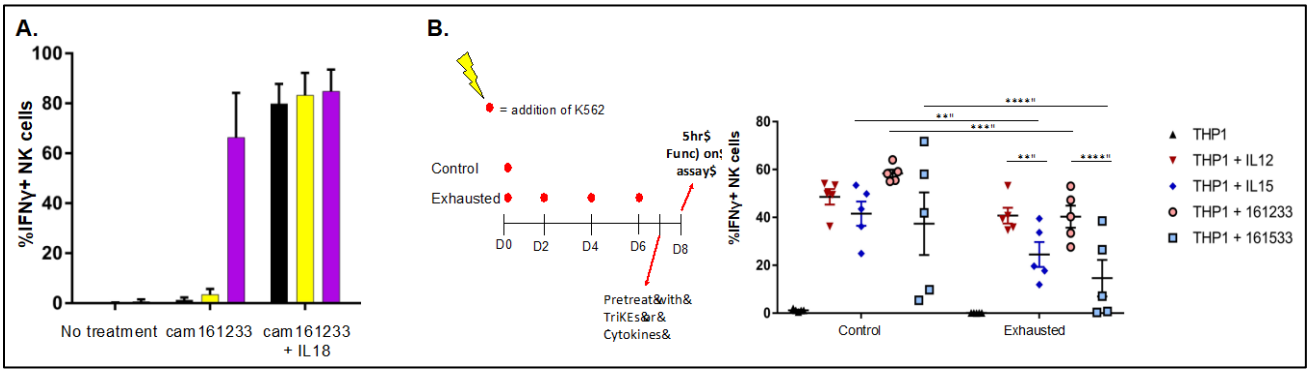

Figure 4: IL-12 TriKE improves NK cell inflammatory responses and rescues NK cell exhaustion

In terms of training activities and professional development the project provided me the ability to attend two premier conferences. The first, the 2018 AACR (American Association of Cancer Research) meeting held

in Chicago in April, focuses primarily on general cancer research. I attended a large number of talks focusing on tumor biology and immunotherapy. At this meeting I gave a talk focusing on the mechanism of action of the improved cam161533 TriKE. The second meeting I attended was the 2018 SNI (Society of Natural Immunology) meeting held in San Antonio in May. This meeting focuses primarily on NK cell biology, and all the eminent researchers on the field attend it. I presented a poster on TriKEs, focusing again on the cam161533 TriKE, while one of my graduate students presented on the cam1615CLE12A TriKE. Besides the meetings the project has provided protected time allowing me to attend several local seminars, including the cancer center seminar and the Garibaldi lecture at the University of Minnesota. The protected time also allowed me to recently author several publications related to this project (reference listed below).

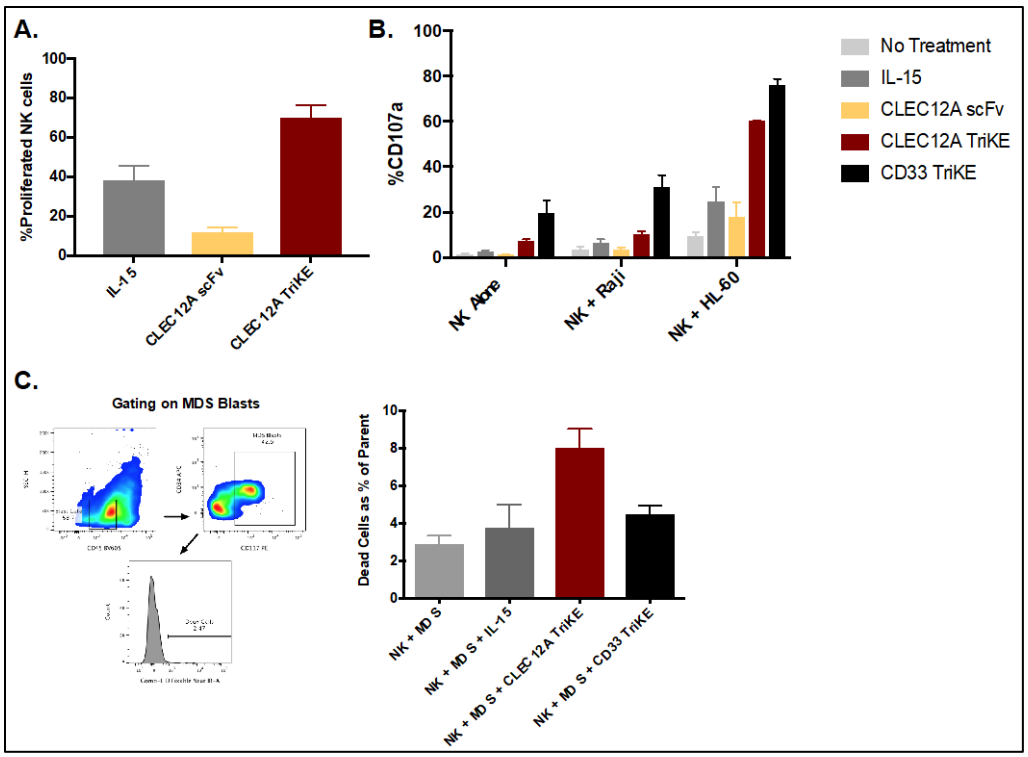

Figure 5: CLEC12A TriKE induces specific targeting of myeloid malignancies

- **Felices M**, Lenvik AJ, McElmurry R, Chu S, Hinderlie P, Bendzick L, Geller MA, Tolar J, Blazar BR, Miller JS. Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI Insight. 2018 Feb 8;3(3). pii: 96219. doi: 10.1172/jci.insight.96219. [Epub ahead of print] PubMed PMID: 29415897; PubMed Central PMCID: PMC5821201.
- Uppendahl LD, Dahl CM, Miller JS, **Felices M**, Geller MA. Natural Killer Cell-Based Immunotherapy in Gynecologic Malignancy: A Review. Front Immunol. 2018 Jan 5;8:1825. doi: 10.3389/fimmu.2017.01825. eCollection 2017. Review. PubMed PMID: 29354116; PubMed Central PMCID: PMC5760535.
- Sarhan D, Brandt L, **Felices M**, Guldevall K, Lenvik T, Hinderlie P, Curtsinger J, Warlick E, Spellman SR, Blazar BR, Weisdorf DJ, Cooley S, Vallera DA, Önfelt B, Miller JS. 161533 TriKE stimulates NK-cell function to overcome myeloid-derived suppressor cells in MDS. Blood Adv. 2018 Jun 26;2(12):1459-1469. doi: 10.1182/bloodadvances.2017012369. PubMed PMID: 29941459; PubMed Central PMCID: PMC6020813.
- Don Yun H, **Felices M**, Vallera DA, Hinderlie P, Cooley S, Arock M, Gotlib J, Ustun C, Miller JS. Trispecific killer engager CD16xIL15xCD33 potently induces NK cell activation and cytotoxicity against neoplastic mast cells. Blood Adv. 2018 Jul 10;2(13):1580-1584. doi: 10.1182/bloodadvances.2018018176. PubMed PMID: 29980573; PubMed Central PMCID: PMC6039654.

During the next reporting period we plan to finish up and submit manuscripts for projects on the IL-12, IL-21, NKG2C, PD-L1, and CLEC12A TriKEs. We also hope to optimize and validate 41BBL, NKG2A and KIR TriKEs, albeit the latter two have proved difficult, as explained. We will also submit a manuscript for the he new camelid platform (cam161533) by the end of the year.

4. Impact (Year-2):

The goal of the proposed work is to significantly improve cellular therapies against chronic myeloid leukemia (CML), covered in the FY15 Myeloproliferative disorders topic area, and related myeloid disorders acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). CML has an incidence of 1-2 cases per 100,000 adults and accounts for about 15% of newly diagnosed cases of leukemia in adults. Prognosis for CML has significantly improved due to the advent of first, second, and third-generation tyrosine kinase inhibitors (TKIs), yet there is still worse survival in the elderly and alternative approaches remain necessary for patients who fail TKI treatment (due to TKI resistant mutation) or patients that present with advanced CML. CML and MDS can progress into AML, which predicts a worse prognosis and a requirement for transplantation as the only curative therapy. Even with transplantation, which is a high-risk procedure and is not well tolerated by elderly patients, the risk for relapse still remains high with current therapeutic options. As the population ages the incidence of AML, MDS, and CML cases increases. Cellular therapy that specifically targets the myeloid tumor cells by the immune system would be of great value independently at earlier stages of disease and as a complementary approach to current therapies at later stages. This type of therapy has the potential to be utilized on its own, depending the stage of intervention, reducing the need for toxic chemotherapeutic and radiation therapy intervention.

The immunotherapeutic value of NK cells is currently being exploited against myeloid malignancies in a variety of ways. NK cells can drive tumor killing in the allogeneic hematopoietic cell transplant (HCT) or NK cell infusion setting, where the graft versus leukemia (GVL) effect is driven through KIR-HLA ligand mismatch, preventing attenuation of NK cell activation through inhibitory KIR. KIR blocking monoclonal antibodies (mAbs) are currently being tested in the clinic to drive this effect in the autologous setting. To drive NK cell antibody-dependent cell-mediated cytotoxicity (ADCC) against myeloid tumors anti-CD33 mAbs have been tested, but results haven't been promising with use of the antibody on its own. Another approach to enhance NK cell immunotherapy is through cytokine treatment, both in the pre- and post-transplant settings. Although this approach is promising, off target effects are a major concern. One final approach generating excitement in the cellular immunotherapy field, also shown to enhance NK cell function, is disruption of the PD-1/PD-L1 checkpoint blockade axis through use of blocking mAbs.

The novelty of the TriKE molecules being proposed here is that they group several of the signals discussed at the NK/myeloid tumor synapse, rather than just focusing the therapy on one signal. The TriKE molecules contain targeting to myeloid cells, through an CD33 scFv or an anti-CLEC12A scFv, and a potent NK cell activation signal, through either a humanized single domain anti-CD16 (cam16, formerly occupied by anti-CD16 scFv) or an anti-NKG2C scFv. Direct binding of CD16 is thought to cause a stronger interaction, and enhanced cytotoxicity, than binding of the low affinity CD16 receptor to the Fc portion of an antibody during standard ADCC and this signal has been further improved (about 28-fold) through the cam16 addition. Besides the targeting and primary activation signals, some of the TriKEs proposed generate a separate biologically relevant function through the third signal. The PD-L1-TriKE prevents PD-1 mediated suppression of NK cell function. The IL-12-TriKE is meant to drive NK cell mediated inflammation. The IL-21-TriKE will induce targeted proliferation of the NK cells without inducing senescence. And finally, the 41BBL-TriKE drives activation/co-stimulation through a second activating receptor. All of theses biologically relevant functions are independent and will induce enhanced function in a variety of settings. Ideally, they can help jumpstart functionality of the patient's own NK cells through enhancing activating signals or blocking inhibitory signals derived from the tumor. In scenarios where myeloid blasts have taken over and/or tumor burden is too high, the TriKEs would have to be utilized in addition to hematopoietic cell transplant and perhaps other standard therapies. However utilization of the TriKEs in this setting would help reduce the risk of relapse, which is quite high in some myeloid disorders. Success with any of the TriKEs, measured by a significant increase in NK cell function against primary blasts over the BiKE (or no construct) during the proposal, would have to be validated in a humanized mouse models prior to testing the molecule in a clinical setting. Given the way the TriKE molecules integrate several signals at the NK/tumor cell synapse, they have the potential to achieve similar immunotherapeutic success to that seen by chimeric antigen receptor (CAR) approaches. The big difference is that they would do this in a more economical "off-the shelf" manner that does not require individualized gene therapy and can be applied to a large number of people. This would represent a major advance in NK cell based immunotherapies against tumors, significantly driving the field forward.

5. Changes/Problems (Year-2):

As discussed in the accomplishments section, creation of two of the inhibitory TriKEs, the anti-KIR (SA1.1) and the anti-NKG2A (SA1.2) TriKEs, has proven difficult due to expression (NKG2A) and binding specificity (KIR) issues. While we are still trying to optimize these, we are not confident that the optimization will be successful. Thus we have switched our efforts in four ways: 1) we have moved up production and testing of the IL-12 (SA2.1) and IL-21 (SA2.2) TriKEs, which seem to be working well; 2) we have focused a good amount of effort in improving the CD16 engaging portion of the platform which should have important effects on clinical implementation of the TriKE; 3) we have started projects activating NKG2C instead of CD16; and 4) we have started projects targeting CLEC12A instead of CD33 to improve myeloid tumor targeting. As expected, science is fluid and we are moving forward with what works and getting away from TriKEs that don't work. We believe these changes will result in several publications as well as increased translational impact.

6. Products (Year-2):

- **Felices M**, Lenvik AJ, McElmurry R, Chu S, Hinderlie P, Bendzick L, Geller MA, Tolar J, Blazar BR, Miller JS. Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI Insight. 2018 Feb 8;3(3). pii: 96219. doi: 10.1172/jci.insight.96219. [Epub ahead of print] PubMed PMID: 29415897; PubMed Central PMCID: PMC5821201.
- Uppendahl LD, Dahl CM, Miller JS, **Felices M**, Geller MA. Natural Killer Cell-Based Immunotherapy in Gynecologic Malignancy: A Review. Front Immunol. 2018 Jan 5;8:1825. doi: 10.3389/fimmu.2017.01825. eCollection 2017. Review. PubMed PMID: 29354116; PubMed Central PMCID: PMC5760535.
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7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS (Year-2)

Name	Martin Felices
Project Role	PD/PI
Researcher Identifier (ORCID)	0000-0002-5945-0634
Nearest person month worked	4 calendar months
Contribution to Project	Experimental design, data analysis, drug design, project direction
Funding Support	See attached support page.

Name	Behiye Kodal
Project Role	Researcher 3 (Scientist)
Researcher Identifier (ORCID)	N/A
Nearest person month worked	6 calendar months
Contribution to Project	Experimental execution, data analysis
Funding Support	N/A

Name	Alexander Lenvik
Project Role	Researcher 3 (Scientist)
Researcher Identifier (ORCID)	N/A
Nearest person month worked	6 calendar months
Contribution to Project	Experimental execution, data analysis, drug design
Funding Support	N/A

PREVIOUS/CURRENT/PENDING SUPPORT:

MARTIN FELICES

1. Previous

R01HL122216 (PI: Jeff Miller)

Title: Inducing NK cells to remember and fight cancer

Effort/Salary: 5%

Agency: NIH/NHLBI

Duration: 12/01/2014-11/30/2018

Funding: $258,745

Grants Officer: Nahed El Kassar, nahed.elkassar@nih.gov

Goal: This program will improve transplant outcome by taking account of NK cell receptor genes to select donors that will reduce relapse of leukemia and increase survival of the transplant recipient.

Specific aims: Aim 1: Investigate the unique microRNA profile of adaptive NK cells and requirements for their expansion.
Aim 2: Determine which signaling and transcription factor networks promote the Development and survival of HCMV-induced adaptive NK cells.
Aim 3: In vivo analysis of adaptive NK cell survival, function and homing in a xenogeneic model of adoptive transfer of human NK cells and HCMV reactivation.

Overlap: None

T32HL007062 (PI: Vercellotti)

Title: Hematology Research Training Program

Effort/Salary: 100%

Agency: NIH

Duration: 06/01/2011-05/31/2013 (supported)

Funding: $437,036 (current annual direct)

Grants Officer: Manda C. Richards, Grants Management Specialist
301.827.7977
manda.richards@nih.gov

Goals: This training program is part of University of Minnesota Department of Medicine, Division of Hematology, Oncology, and Transplantation. It is focused on training MDs and PhDs for academic careers that emphasize Hematology-related Research.

Specific aims: N/A

Overlap: None

2. Current

W81XWH-16-1-0380/0010866661-0001(PI: Felices)

Title: CA150085 - Enhancing Natural Killer Cell-Mediated Targeting and Responses to Myeloid Leukemias

Effort/Salary: 30%

Agency: U.S. Department of Defense

Duration: 09/30/2016-09/29/2019

Funding: $359,979

Grants Officer: Elayne Seiler, Grants Specialist
elayne.k.seiler.civ@mail.mil
301.619.7358

Goal: Generate TriKEs targeting pathways involved in suppression of NK cell function and generate TriKEs targeting pathways involved in amplifying NK cell function.

Specific aims: 1. Generation of TriKes targeting pathways involved in suppression of NK cell function
2. Generation of TriKes targeting pathways involved in amplification of NK cell function

Overlap: None

R21CA216652 (PI: Moriarity)

Title: Genetically Modified Natural Killer Cells for Cancer Immunotherapy

Effort/Salary: 5%

Agency: NIH

Duration: 09/06/2018-08/31/2020

Funding: $159.420

Grants Officer: Anthony Welch
awelch@ncifcrf.gov

Goal: We will utilize a specific immune cell, termed Natural Killer cells, and modify their genetic code to enhance their ability to treat a pre-existing cancer.

Specific aims: Specific Aim 1: Optimize enrichment and expansion of genetically modified primary human NK cells
Specific Aim 2: Functionally test knockout and/or gene edited primary human NK cells for enhanced expansion, survival, and/or cancer cell killing in vitro and in vivo

Overlap: None

P01CA065493 (PI: John Wagner)

Title:	Biology and Transplantation of the Human Stem Cell
Effort/Salary:	10%
Agency:	NIH/NCI
Duration:	07/01/2015-06/30/2020
Funding:	$192,000
Grants Officer:	William Merritt, PhD merrittw@mail.nih.gov
Goal:	The main goal of this project is to understand how to exploit NK cell therapy along with umbilical cord blood transplantation.
Specific aims:	Cancer Center Project 3 (Jeff Miller), Project Title: Adaptive NK Cell Therapy to Improve UCB Transplant Outcomes 1. Evaluate the clinical impact of IL-15 signaling on adaptive NK cells. CMV reactivation, which occurs in 50% of CMV seropositive patients undergoing UCBT, induces a unique population of adaptive NK cells and is correlated with protection from relapse. In SA1.1, we will perform high-resolution phenotypic and functional analyses of blood from UCB transplant patients to test our hypothesis that expansion of specific subsets of adaptive NK cells lacking proximal signaling components (EAT-2, SYK, FceR1g) mediate relapse protection and enhanced survival after UCBT. We will also determine the mechanism by which these cells differentiate in response to IL-15 signaling. In SA1.2, we will perform two clinical trials in patients with hematologic malignancies undergoing UCBT after non-myeloablative conditioning (NMAC) whom have high risk of relapse (~35%). The primary objective of these trials is to safely induce expansion of activated adaptive NK cells. The secondary clinical endpoint is relapse. The first trial will test the hypothesis that IL-15 signaling through trans-presentation by IL-15Ra can drive in vivo expansion of endogenous adaptive NK cells early (~42 days) after UCBT, particularly in the context of CMV reactivation. In the second trial, we will add ex vivo manipulation of a day ~42 apheresis product (autologous to the graft but allogeneic to the recipient) based on preliminary data showing that 16-hour ex vivo stimulation of NK cells with IL-15, IL-12, and IL-18 results in superior anti-tumor activity in vitro. 2. Activation and antigen targeting of NK cells for prevention of relapse. In SA2.1, we will pursue two pre-clinical strategies to enhance NK cell function: in vivo NK cell targeting and short-term ex vivo activation of NK cell products prior to infusion. In SA2.1, we will use a xenogeneic model of primary human AML to evaluate human NK cells targeted in vivo with 1) IL-15/IL-15Ra-Fc and anti-CD16x33 BiKE; 2) IL-15/IL-15Ra-Fc-anti-CD33 complexes; or 3) IL-15/IL-15Ra-Fc and anti-CD16xPR1 BiKE to establish the best approach to make NK cells antigen specific. We will also definitively characterize the role of adaptive NK cells, and test modifications to ex vivo activation (IL-15/12/18) proposed in SA1 such as CD16 ligation as suggested by preliminary data. In SA2.2 we will perform a clinical trial to test NK cells targeted with BiKEs or IL-15 complexes in patients with CD33+ and PR1+ malignancies (AML/MDS/CML) that have received non-myeloablative UCBT. After establishing safety, the trial will be powered to detect a reduction of 50% in the primary outcome of relapse.
Overlap:	None

P01CA111412 (PI: Jeff Miller)

Title:	NK cells, their receptors, transplantation and cancer therapy
Effort/Salary:	10%
Agency:	NIH/NCI
Duration:	04/05/2016-03/31/2021
Funding:	$1,416,372
Grants Officer:	William Merritt, merrittw@mail.nih.gov
Goal:	This program will improve transplant outcome by taking account of NK cell receptor genes to select donors that will reduce relapse of leukemia and increase survival of the transplant recipient.
Specific aims:	Aim 1: Understand how KIRs interact with class I HLA to determine NK cell function Aim 2: Test the hypothesis that trans-presentation of IL-15 is needed to optimally expand NK cells in vivo Aim 3: Determine now hCMV induces the development of adaptive NK cells with enhanced ADCC capacity and memory-like properties. Aim 4: Perform clinical trials to exploit enhanced NK cell activation with specific antigen targeting with bi-specific killer engagers (BiKE) to enhance anti-tumor potency of NK cells
Overlap:	None

R35CA197292-02 (PI: Jeff Miller)

Title:	Viral Priming and Targeting NK Cells Against Solid Tumor Malignancies
Effort/Salary:	5%
Agency:	NIH/NCI
Duration:	08/05/2015-07/31/2022
Funding:	$600,000
Grants Officer:	Anthony R. Welch, awelch@ncifcrf.gov
Goal:	The overarching goal is to develop strategies to enhance the anti-tumor activity of endogenous NK cells in patients with solid tumor malignancies. The objective is to develop "off the shelf" reagents to activate NK cells, overcome inhibitory receptor signaling, and target them to specific tumor antigens.
Specific aims:	Aim 1: Use genetic epidemiologic studies to determine whether NK cells “adapted” by viral exposure (cytomegalovirus [CMV] ± human papilloma virus [HPV]) influence the risk of cancer development and response to therapy. Aim 2: Characterize RHAMM as a promising target to eliminate tumor cells and Immunosuppressive non-malignant cells from the tumor microenvironment, Aim 3: Test whether newly discovered “adaptive” NK cells mediate enhanced anti-tumor activity via CD16 signaling. Aim 4: Develop and test novel agents to target activated NK cells against solid tumors.

Overlap: None

P30CA077598 (PI: Douglas Yee)

Title: Cancer Center Support Grant

Effort/Salary: 10%

Agency: NIH/NCI

Duration: 06/01/1998-01/31/2019

Funding: $2,342,880

Grants Officer: Sonya Roberson, robersos@mail.nih.gov

Goal: The goal of this grant is to provide support for the University of Minnesota's NCI designated Comprehensive Cancer Center to provide shared resources to its research investigators and service to the community in providing public and professional cancer education, outreach, and cancer information.

Specific aims: N/A

Overlap: None

RSG-14-151-01-CCE (PI: Geller)

Title: Natural Killer Cell Immunotherapy for Ovarian Cancer

Effort/Salary: 5%

Agency: American Cancer Society

Duration: 01/01/2015-12/31/2018

Funding: $798,851

Grants Officer: The American Cancer Society
Extramural Grants Department
250 Williams Street NW, 6th Floor
Atlanta, GA 30303

Goal: We are developing an anti-mesothelin chimeric antigen receptor (CAR) in iPSCs to produce a targeted NK cell population effective against ovarian cancer, where 70% of tumors express mesothelin. Our goal is for CAR-expressing NK cells to be used as a readily available, "off-the-shelf" product for anti-tumor immunotherapy.

Specific aims: 1. Express an anti-mesothelin chimeric antigen receptor (CAR) in human induced pluripotent stem cells (iPSCs) to create targeted NK cells with increased ability to kill human ovarian cancer cells.
2. Evaluate in vivo anti-ovarian cancer activity of NK cells derived from human iPSCs expressing anti-mesothelin chimeric receptors.

Overlap: None

N/A (PI: Miller)

Title: Next Generation TriKE Development

Effort/Salary: 21%

Agency: GT Pharma

Duration: 10/01/2016-12/31/2019

Funding: $1,737,326 (total costs)

Grants Officer: Raymond Urbanski, Chief Medical Officer: rwu@gtbiopharma.com

Goal: The primary rationale for this project is to build on our existing TriKE platform.

Specific aims: (1) The first major goal is to further develop and test TriKEs that are specific for EpCAM, CD133, EpCAM and CD133 (TetraKE), Her2 and Mesothelin as outlined in the licensing plan.

a) Each of these TriKEs will require an optimized IL-15 linker and scFv against CD16 consistent with our ability to protect IP and freedom to operate.
b) Each of these TriKEs will require testing on patients cells to identify cohorts amenable to TriKE therapy.

(2) The second goal is to explore higher production systems that generate higher concentrations of TriKEs for expanded use beyond the initial bacterial production used for phase I testing.

Overlap: None

3. Pending

None