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TITLE: Development of Epitope-Focused Tumor Vaccine to Prevent Escape from Immune Surveillance by the NKG2D Pathway

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14. ABSTRACT

MICA is a stress proteins that is frequently expressed by many different types of human cancers due to genomic damage and aberrant signaling. Expression of MICA marks stressed/infected cells for elimination by cytotoxic lymphocytes, in particular NK cells and CD8 T cells. Engagement of the NKG2D receptor by MICA triggers NK cell mediated cytolysis and provides a costimulatory signal in T cells. Many cancers evade this important pathway through proteolytic shedding of MICA. Shed MICA is associated with progression in many human solid and hematological malignancies. The shedding process targets the membrane-proximal alpha3 domain of MICA through the coordinated action of the disulfide isomerase ERp5 and several proteases. MICA antibodies from patients responding to an autologous cancer vaccine combined with CTLA-4 blockade were found to sterically inhibit shedding through binding to the MICA alpha3 domain. The goal of this project was to develop a novel cancer vaccine which inhibits this important immune evasion mechanism. The specific aims of this proposal were to 1. Generate MICA alpha3 domain vaccine, 2. Test the therapeutic activity of vaccine in melanoma mouse model, 3. Investigation of immunological mechanisms leading to protective effect of the vaccine. Preliminary data show that a vaccine targeting the MICA alpha3 domain indeed induces polyclonal antibodies that strongly inhibit MICA/B shedding by cancer cells. This vaccine has significant efficacy in a mouse model of melanoma in controlling experimental lung metastasis and subcutaneous tumor growth. Dissection of the immunological mechanisms showed both CD8 T cells and NK cells to be important for the protective effect of the vaccine. This vaccine could be used to prevent the emergence of metastases in high-risk patients, for example in patients following surgical removal of a locally advanced melanoma who are at a high-risk for disease recurrence.

15. SUBJECT TERMS

None listed

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1. INTRODUCTION:

MICA and MICB (MIC) are stress-induced NKG2D ligands which are frequently upregulated in tumor cells due to genomic damage. MIC expression flags tumor cells for elimination by cytotoxic lymphocytes it activates the cytotoxic function of NK cells and provides a critical costimulatory signal for cytotoxic T cells. However, many advanced human tumors escape from NKG2D-mediated immune surveillance by proteolytic shedding of MIC from the tumor cell surface, a process in which the alpha3 domain of MIC is targeted first by the disulfide isomerase ERp5 and subsequently by metalloproteases including ADAM10 and ADAM17. Shed MICA downregulates NK cell and T cell function by triggering internalization and degradation of the NKG2D receptor. Melanoma patients treated with autologous cancer vaccine (GVAX) and CTLA-4 blockade antibody (Ipilimumab) were found to develop antibodies specific for MICA. Long-term responders were found to have lower circulating shed MICA (sMICA) levels and antibodies to MICA. Preliminary results from our lab have demonstrated that patient-derived MICA specific antibodies improve tumor immune surveillance by inhibiting MICA shedding and increasing surface MICA levels on tumor cells. Human mAbs isolated from these patients bind to the MIC alpha3 domain and exhibited significant therapeutic efficacy in animal models. We therefore proposed to develop a novel cancer vaccine based on the MIC alpha3 domain that induces endogenous antibodies which inhibit MIC shedding from the tumor cell surface. Such a vaccine could provide long-term protection against tumor escape from this important immune pathway.

2. KEYWORDS:

MICA, vaccine, melanoma, polyclonal antibodies, mesoporous silica rods, NKG2D, CD8 T cells, NK cells

3. ACCOMPLISHMENTS:

Major goals of the project

Specific Aim 1: Generation of MIC alpha3 domain vaccine	Proposed Timeline	Date of completion	Percent completion
Major Task 1: Production of MICA alpha3 ferritin vaccine	Months		
Subtask 1: Expression of MICA-ferritin fusion protein in insect cells and downstream purification of the protein from the supernatant. MICA alpha3 ferritin fusion proteins (abbreviated as MICA-ferritin) will be expressed in Sf9 insect cells using the Baculovirus system. The protein will be purified by HA-affinity chromatography using an epitope tag placed at the N-terminus of the protein; aggregates will be removed by size exclusion chromatography. Protein purity will be verified by SDS-PAGE.	1	August 2016	100 percent
Subtask 2: Formulating the vaccine by loading the MICA-ferritin protein or ovalbumin protein (control) along with GM-CSF and CpG oligonucleotide onto Mesoporous silica rods (MSR). The MICA-ferritin fusion or a control protein (ovalbumin) will be absorbed to MSR for 12 hours at room temperature. The particles will then be lyophilized and stored for subsequent *in vivo* analysis.	1	August 2016	100 percent
Milestone(s) Achieved: Generation of MICA-ferritin vaccine for carrying out *in vivo* immunization and tumor challenge experiments.	1	August 2016	100 percent
Specific Aim 2: Assess therapeutic activity of the MICA-ferritin vaccine			

Major Task 2: Optimization of vaccine dose and timing of tumor challenge			
Subtask 1: Local IRB/IACUC Approval	Jan 2016-April 2016	April 2016	
Subtask 2: HRPO/ACURO Approval	April 2016-Aug 2016	August 2016	
Subtask 3: Optimize the amount of MICA-ferritin protein utilized in the vaccine (5-200 µg) by injecting the vaccine subcutaneously into the flank of C57BL/6 mice (n=4 in each group), collecting the sera at weekly intervals and measuring the antibody titers by ELISA and flow cytometry.	2	October 2016	100 percent
Subtask 4: Challenge MICA-ferritin immunized mice with intravenous injection of B16F10 cells expressing MICA on day14 to determine if the mice challenged with tumor cells without booster immunization are protected. Alternatively, mice will receive a booster immunization at the time of tumor challenge. Naïve mice and ovalbumin protein immunized mice will be used as control groups (n=6 in each group).	3	December 2016	100 percent
Milestone(s) Achieved: Local IRB/IACUC and HRPO/ACURO Approval prior to the start of the funding (September 2016). The dose of the vaccine and whether or not a boost is required for the beneficial effect of the vaccine will be defined.	4	December 2016	100 percent
Major Task 3: Testing the effect of MICA alpha3 vaccine in a therapeutic and prophylactic setting			
Subtask 1: Local IRB/IACUC Approval	Jan 2016-April 2016	April 2016	100 percent
Subtask 2: HRPO/ACURO Approval	April 2016-Aug 2016	August 2016	100 percent
Subtask 3: Testing the effect of MICA-ferritin vaccine in a prophylactic setting by challenging the immunized mice with intravenous injection of B16F10 tumors expressing human MICA. Naïve mice and ovalbumin protein immunized mice will be used as control groups (n=8 in each group) and subsequently challenged with tumors.	5	January 2016	100 percent
Subtask 4: Testing the effect of MICA-ferritin vaccine in a prophylactic setting by challenging the immunized mice with subcutaneous injection of B16F10 or B16F1 tumors expressing human MICA. Naïve mice and ovalbumin protein immunized mice will be used as control groups (n=8 in each group) and subsequently challenged with tumors.	5	January 2016	100 percent

Subtask 5: The protective effect of MICA-ferritin vaccine will be tested by implanting B16F10 tumors and subsequent surgical removal advanced primary tumors. Mice will then be immunized and their survival monitored. Histological analysis will document the number of metastases in MICA-ferritin and ovalbumin immunized mice (n=10 in each group)	5-6	February 2016	100 percent
Milestone(s) Achieved: Local IRB/IACUC and HRPO/ACURO Approval prior to the start of the funding (September 2016). We will have an understanding of effect of MICA-ferritin vaccine in controlling and preventing tumor growth and metastasis in the immunized mice.	7	February 2016	100 percent
Specific Aim 3: Investigation of immunological mechanism			
Major task 4: Investigate the immunological mechanisms induced by MICA-ferritin vaccine			
Subtask 1: Local IRB/IACUC Approval	Jan 2016-April 2016	April 2016	100 percent
Subtask 2: Local IRB/IACUC Approval	April 2016-Aug 2016	August 2016	100 percent
Subtask 3: Determine the cellular mechanism of action in lung metastasis and subcutaneous tumor model- NK cells or CD8 T cells will be depleted using specific antibodies in the MICA-ferritin and ovalbumin immunized mice prior to tumor challenge experiment (outlined in subtasks 3 and 4 under major task 3). The individual role of these cell populations in conferring immunity in the lung metastasis model be determined (n=8 in each group).	7-8	April 2017	100 percent
Subtask 4: Determine the requirement for NKG2D pathway in therapeutic setting by performing the tumor challenge experiment (outlined in subtask 4 under major task 3) in NKG2D knockout mice immunized with MICA-ferritin and ovalbumin (control) vaccine and comparing the data with wild type mice immunized with the proteins (n=8 in each group).	9	May 2017	25 percent
Subtask 5: To determine if there is antibody-dependent cytotoxicity induced by NK cells or macrophages upon MICA-ferritin vaccination, tumor challenge experiments (outlined in subtask 4 under major task 3) will be performed in Fcgr3 and Fcer1g knockout mice immunized with MICA-ferritin and ovalbumin (control) vaccine (n=8 in each group).	10	June 2017	25 percent
Subtask 6: The role of MICA-ferritin vaccine in inducing long term protective immunity will be confirmed by challenging the surviving mice from the metastasis model with a high dose of intravenous tumor cells with or without MICA expression. Survival of these mice will be monitored.	10-11	July 2017	25 percent
Milestone(s) Achieved: Local IRB/IACUC and HRPO/ACURO Approval prior to the start of the funding (September 2016). The	12	July 2017	

immunological mechanisms induced by MICA-ferritin vaccine leading to therapeutic and protective benefits against melanoma tumors will be well characterized.			50 percent

What was accomplished under these goals?

Specific Aim 1: Generation of MIC alpha3 domain vaccine.

Major Task 1: Production of MICA alpha3 ferritin vaccine.

1. Expression of MICA-ferritin fusion protein and downstream purification.

The presence of MICA antibodies in patients receiving GVAX plus Ipilimumab demonstrated that MICA proteins are immunogenic in humans, even in the context of a cell-based tumor vaccine. We designed a protein-based vaccine that focuses the antibody response on the MICA alpha3 domain, with the goal of inducing high-titer polyclonal antibodies that strongly inhibit MICA/B shedding. The MICA/B alpha1-alpha2 domains were intentionally omitted to prevent induction of antibodies that block NKG2D receptor binding. The MICA/B alpha3 domains are highly conserved (94.2% sequence identity for the common MICA*009 and MICB*005 alleles). A substantial body of work in the vaccine field has demonstrated that display of a protein domain on the surface of a viral capsid or similar nanoparticle greatly increases its immunogenicity. We therefore generated a self-assembling protein nanoparticle that displays the MICA alpha3 domain. The MICA alpha3 sequence was linked to the N-terminus of ferritin which forms nearly spherical particles composed of 24 subunits arranged with octahedral symmetry (**Fig. 1A**). The *Helicobacter pylori* ferritin sequence was selected because it is highly divergent from mammalian ferritins. These protein nanoparticles thus display 24 copies of the MICA alpha3 domain for efficient crosslinking of the B cell receptor. A HA epitope tag was attached to the N-terminus for efficient affinity purification. The construct was transfected into the Sf9 cells using baculovirus expression system. The MICA alpha3 – ferritin nanoparticles were secreted at a high level by Sf9 cells, and transient transfection resulted in yields of ~15mg/liter. The protein was purified by HA affinity and gel filtration chromatography, and electron microscopy (**Fig.1B)** demonstrated the presence of nanoparticles of the expected size. The purity of the protein was confirmed by SDS-PAGE (**Fig. 1C**).

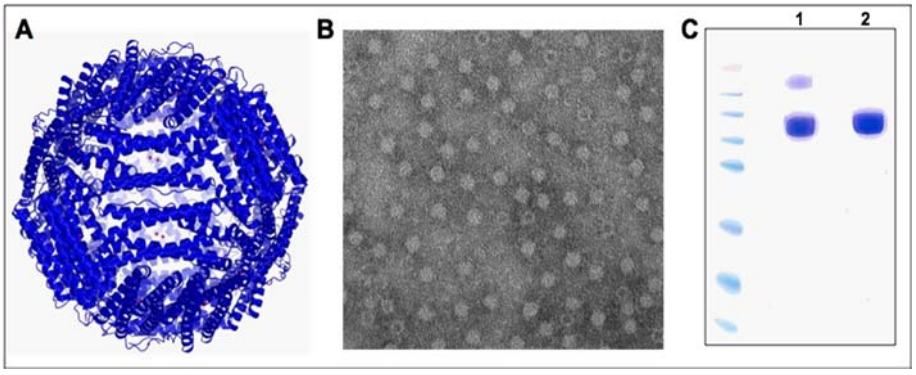


Figure 1. Design of protein nanoparticle that displays the MICA alpha3 domain. A. Crystal structure of ferritin. **B.** Electron microscopy picture of MICA alpha3 ferriting nanoparticles at 98,000-fold magnification. **C.** SDS-PAGE of final product under non-reducing (lane 1) and reducing (2) conditions.

2. Formulation of MICA-ferritin vaccine.

The vaccine was formulated with a scaffold recently designed by our collaborator Dr. David Mooney. His innovative approach creates a microenvironment at the injection site to which large numbers of immune cells are recruited (**Fig. 2**). Mesoporous silica rods (MSR) are injected subcutaneously where they assemble into a macroporous structure as the injection buffer diffuses away.

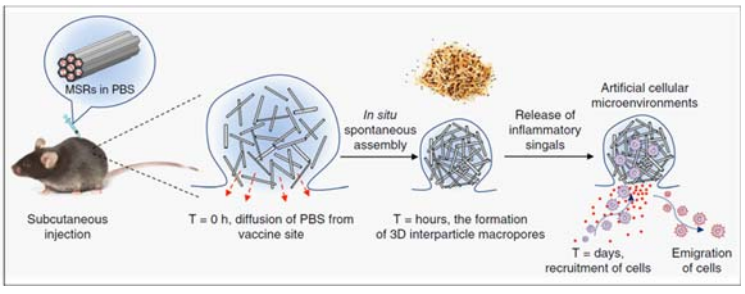


Figure 2. Scaffold system developed by the Mooney lab. The mesoporous silica rods provide a large surface area for absorption and slow release of proteins and adjuvants.

Dendritic cells are recruited to this site by release of GM-CSF, which are exposed within the scaffold to the antigen and adjuvant (CpG oligonucleotide). Following maturation, the dendritic cells migrate to lymph nodes

where they induce a coordinated T cell and B cell response. The vaccine was formulated with 5mg of MSR loaded with 1µg GM-CSF (to recruit dendritic cells), 100µg of CpG oligonucleotide (to induce dendritic cell activation) and 200µg of MICA alpha3-ferritin protein for 12 hours at room temperature, lyophilized, resuspended in PBS and injected subcutaneous into the flank of C57/BL6J mice.

Specific Aim 2: Assess therapeutic activity of the MICA-ferritin vaccine.

Major Task 2: Optimization of vaccine dose and timing of tumor challenge.

1. **Optimize the amount of MICA-ferritin protein utilized in the vaccine.**

The vaccine was formulated with different concentrations of the MICA-ferritin protein (50µg or 100µg or 200µg) along with 5mg of MSR, 1µg GM-CSF and 100µg of CpG oligonucleotide. The vaccine was injected subcutaneously into the flank of C57BL/6 mice (n=4 in each group), sera was collected at weekly intervals and was used for measuring the antibody titers by ELISA using the full length extracellular domain of MICA as capture antigen and detecting the bound MICA specific antibody using HRP conjugated anti-mouse IgG. Immunization of C57BL/6 mice with MICA-ferritin vaccine elicited high and durable end point antibody titers with 200µg protein (**Fig. 3A, red bar**) resulting in the highest antibody titer on all days tested compared to mice immunized with vaccine containing 100µg protein (**Fig. 3A, green bar**), 50µg protein (**Fig. 3A, blue bar**) and 200µg of control protein (**Fig. 3A, black bar**). Flow cytometric analysis was used to assess the binding of serum antibodies to full length MICA expressed on the surface of tumor cells. Briefly, 0.1 million cells were stained with 1µl of serum from non-immunized mice (naïve), mice immunized with control antigen or MICA alpha3 vaccine (vaccinated) in 100µl of PBS for 2 hours. Commercially available monoclonal antibody 6D4 that binds to the alpha1-alpha2 domains of MICA was used as a positive control (10µg). PE conjugated anti-mouse IgG was used as secondary antibody. Staining of B16F10 MICA expressing cells with a small amount (1µl) of day25 post immunization serum from vaccinated mice from the indicated groups in **Fig. 3B** resulted in a high intensity histogram peak for 200 µg>100 µg>50 µg compared to the control groups.

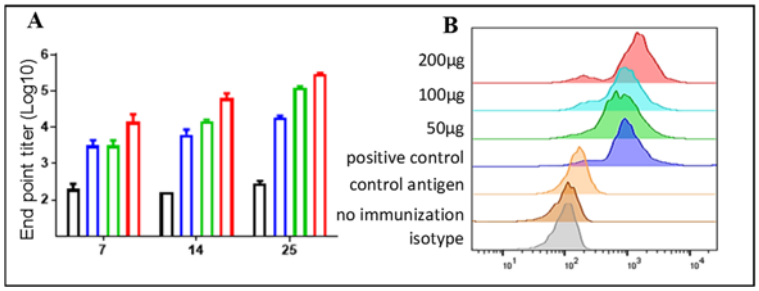


Figure 3. MICA-ferritin vaccine induces high-titer MICA alpha3 specific polyclonal antibodies that bind to MICA on tumor cell surface. A. Elisa to determine the antibody titers in mice vaccinated with MICA vaccine consisting of 50µg or 100µg or 200µg of MICA-ferritin protein along with 5mg of MSR, 1µg GM-CSF and 100µg of CpG oligonucleotide. End point titer is represented on the Y-axis and the serum collection date post immunization is shown on the X-axis. **B.** Histogram peaks showing the binding of MICA alpha3 specific antibodies in the sera of MICA-ferritin immunized mice to full length MICA expressed on the surface of B16F10 mouse melanoma cells when tested by flow cytometry.

In addition to this, *in vitro* functional assays also demonstrated that small quantities of the sera from MICA-ferritin vaccinated mice was sufficient to strongly inhibit MICA shedding by human melanoma cell line (**Figure 4, red bars**) compared to control antigen immunized (**Figure 4, blue bar**) and naïve mice (**Figure 4, grey bar**)

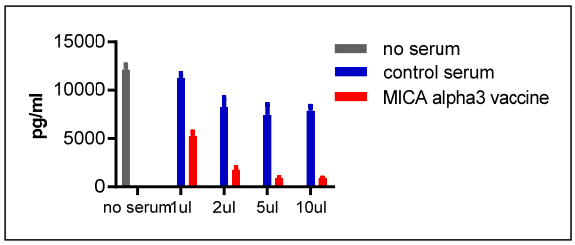


Figure 4. Polyclonal MICA alpha3 domain antibodies strongly inhibit MICA shedding by human melanoma cells. Human A375 melanoma cells were cultured for 24 hours in media +/- sera (1-10 µl) from mice immunized with MICA-ferritin or control antigen. Shed MICA was quantified by sandwich ELISA (ELISA antibodies bind to alpha1-alpha2 domain of MICA and does not interfere with the alpha3 domain).

Major Task 3: Testing the effect of MICA alpha3 vaccine in a therapeutic and prophylactic setting.

1. **Testing the effect of MICA-ferritin vaccine in a prophylactic setting by challenging the immunized mice with intravenous injection of B16F10 tumors expressing human MICA.**

The efficacy of MICA alpha3 vaccine was first tested in a melanoma lung metastasis model. C57BL/6J mice were vaccinated with MICA-ferritin vaccine (200µg of MICA-ferritin protein along with 5mg of MSR, 1µg GM-CSF and 100µg of CpG) or OVA (200µg of MICA-ferritin protein along with 5mg of MSR, 1µg GM-CSF and 100µg of CpG) as control group (6 mice in each group). Following immunization, the mice were challenged by intravenous injection of $0.5*10^6$ B16F10-MICA. Lungs were harvested two weeks following tumor challenge and the number of lung metastases were counted. MICA-ferritin vaccinated mice (red bar) were nearly tumor free. In contrast, non-immunized age-matched control group (naïve, black bar) and mice vaccinated with control antigen (blue bar) had large numbers of lung metastases (average of ~150 lung mets/mouse) (**Fig. 5A**). Importantly, shed MICA was undetectable in sera of mice immunized with MICA alpha3 vaccine (red triangle) while high levels of sMICA were detected within two weeks after tumor challenge in the sera of mice immunized with control antigen (blue square) and the non-immunized group (black circle) (**Fig. 5B**).

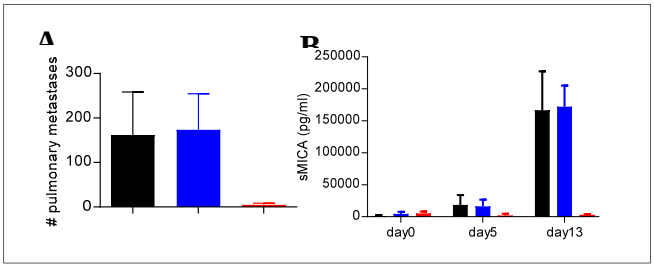


Figure 5. MICA alpha3 vaccine prevents metastases. A. 8 week old C57BL/6J female mice were immunized with MICA alpha3 or ovalbumin (control Ag) vaccine followed by a boost on day 21. Two weeks later, mice were challenged by intravenous injection of $5x10^5$ MICA-expressing B16F10 cells. Mice were euthanized 14 days after tumor challenge and the number of pulmonary metastases quantified. **B.** Shed MICA (sMICA) levels in the sera was monitored by ELISA as described in figure 4.

2. **Testing the effect of MICA-ferritin vaccine in a prophylactic setting by challenging the immunized mice with subcutaneous injection of B16F10 tumors expressing human MICA.**

MICA-expressing B16F10 cells were implanted subcutaneously into the flank of MICA alpha3 vaccinated, control antigen vaccinated or naïve mice (8 mice in each group). Serum was collected prior to tumor inoculation and at weekly intervals. Tumor volume was measured and recorded every other day and the mice were euthanized when tumors reached around $300mm^2$. Tumor growth was significantly delayed in the MICA-ferritin vaccinated group **(Fig. 6A, red circles)** compared to the control group **(Fig. 6A, black circles)**. However, the tumors relapsed by ~day 18 in these mice. Flow cytometric analysis of relapsing tumors showed outgrowth of MICA-negative cells (that probably were not transduced by the lentiviral vector).

Shed MICA was undetectable in sera of mice immunized with MICA alpha3 vaccine **(Fig. 6B, red triangle)** while high levels of sMICA were detected within two weeks after tumor challenge in the sera of mice immunized with control antigen **(Fig. 6B, black triangle)**.

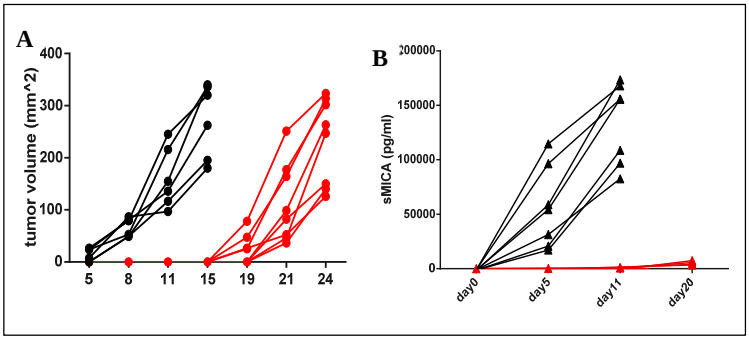


Figure 6 A. 7 week old C57BL/6 female mice (n=8) were immunized with MICA-ferritin vaccine and boosted on day 14. The mice were challenged with subcutaneous injection of 0.5x106 B16F10 cells expressing MICA on day 25 after initial vaccination and the tumor volume was measured every other day. Tumor growth in the MICA-ferritin immunized group was found to be significantly slower (red square) compared to the naïve, untreated age matched control group (black square). **B.** sMICA levels was undetectable in sera of mice immunized with MICA-ferritin vaccine (red triangle) while high levels of sMICA were detected within two weeks after tumor challenge in the sera of the non-immunized control group (black triangle).

3. **Testing the effect of MICA-ferritin vaccine in a therapeutic setting by challenging the immunized mice with subcutaneous injection of B16F10 tumors expressing human MICA.**

C57BL/6 mice immunized with MICA-ferritin vaccine or the control antigen as described above were challenged by subcutaneous injection of doxycycline inducible B16F10-MICA tumor cells. These cell lines do not have any basal MICA expression both *in vitro* and *in vivo* until the addition of doxycycline. Induction of MICA expression

on established tumors (~50-75 mm^2) showed tumor regression specifically in the MICA-ferritin vaccinated group (**Fig. 7A**). Importantly, mice immunized with MICA-ferritin vaccine (red circle) had very low levels of shed MICA compared to mice immunized with control antigen (blue square) and the non-immunized control group (black circle) (**Fig. 7B**).

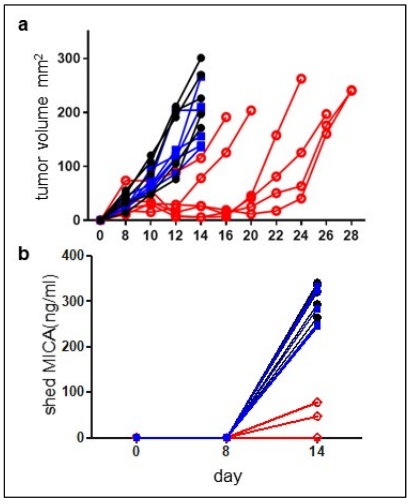


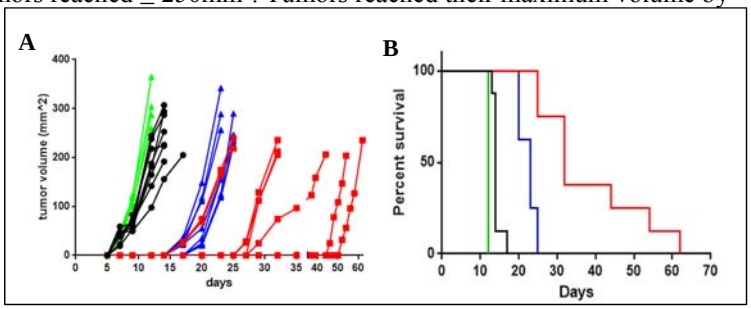
Figure 7. A. 7 week old C57BL/6J mice were immunized with MICA alpha3 vaccine or control antigen and boosted on d21. Doxycycline inducible B16F10 MICA tumors were injected subcutaneously one week post boost and MICA expression was induced on day 8 when the tumors were ~50mm^2. Tumor growth was measured every other day following inoculation. Tumors in the naïve control (black lines) and control antigen immunized group (blue lines) reached their maximum size by day 12, while tumor growth was significantly delayed in the MICA alpha3 vaccinated mice (red lines). **B.** Shed MICA (sMICA) levels in the sera was monitored by ELISA.

Specific Aim 3: Investigation of immunological mechanism contributing towards the protective effect of the vaccine.

Major task 4: Investigate the immunological mechanisms induced by MICA-ferritin vaccine

1. Determine the role of CD8 T cells in subcutaneous tumor model.

Figure 8. A. 7 week old C57BL/6 female mice were immunized with MICA-ferritin vaccine (n=16) or with OVA control vaccine (n=8) and boosted on day 14. The mice were challenged with subcutaneous injection of 0.5x10^6 B16F10 cells expressing MICA on day 21 after initial vaccination. Mice received intravenous injection of 200 µg of anti-CD8 antibody (n=8) or isotype control antibody (n=8) 2 days prior to tumor challenge and twice a week thereafter at a dose of 100 µg per mouse till the study endpoint. Tumor volume was measured every other day. The mice were euthanized when the tumors reached ≥ 250mm^2. Tumors reached their maximum volume by day 12 in CD8 depleted, OVA protein vaccinated control mice (green triangle) and by day 14 in naïve, untreated, non-depleted control group (black circle). CD8 depletion accelerated tumor growth in MICA-vaccinated group (blue traingle) compared to MICA-vaccinated group that received isotype antibody (red square). **B**. Survival analysis of CD8 depletion experiment showing age matched naïve, untreated, non-depleted control group in black, OVA protein vaccinated group in green, MICA-ferritin vaccinated, CD8 depleted in blue and MICA-ferritin vaccinated, isotype antibody injected mice in red line.



2. Determine the role of NK cells in subcutaneous tumor model.

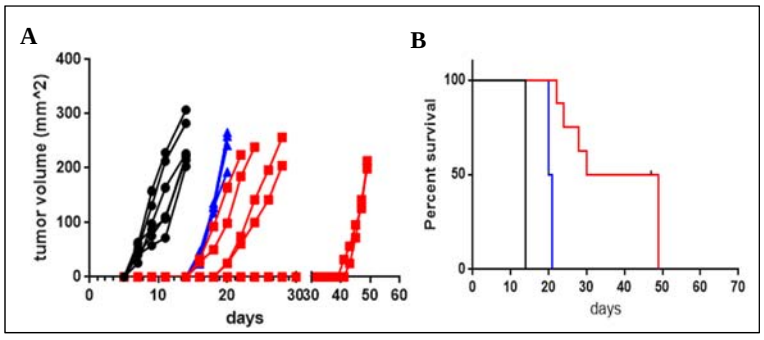


Figure 9 A. 7 week old C57BL/6 female mice were immunized with MICA-ferritin vaccine (n=16) and boosted on day 14. The mice were challenged with subcutaneous injection of 0.5x10^6 B16F10 cells expressing MICA on day 21 after initial vaccination. Mice received intravenous injection of 200 µg of anti-NK1.1 antibody (n=8) or isotype control antibody (n=8) 2 days prior to tumor challenge and twice a week thereafter at a dose of 100 µg per mouse till the study endpoint. Tumor volume was measured every other day. The mice were euthanized when the tumors reached ≥ 250mm^2. Tumors reached their maximum volume by day 14 in naïve, untreated, non-depleted control group (black circle). CD8 depletion accelerated tumor growth in MICA-vaccinated group (blue triangle) compared to MICA-vaccinated group that received isotype antibody (red square). **B**. Survival analysis of NK cell depletion experiment showing age matched naïve, untreated, non-depleted control group in black; MICA-ferritin vaccinated, CD8 depleted in blue and MICA-ferritin vaccinated, isotype antibody injected mice in red line.

3. Role of polyclonal serum antibodies in therapeutic effect of the vaccine.

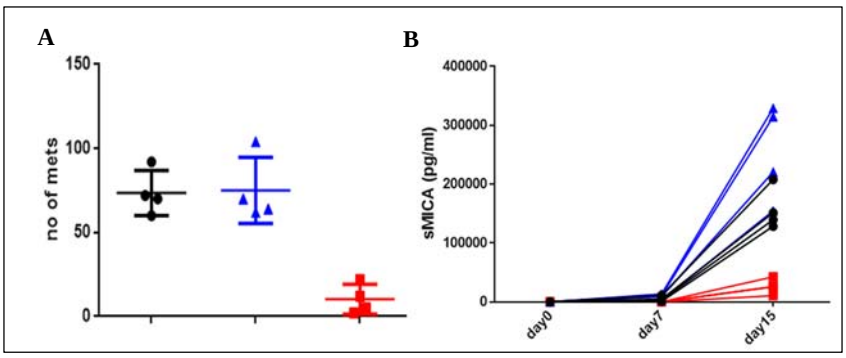


Figure 10 A. 8 week old Ighm^{tm1Cgn}/J (B cell deficient) female mice (n=12) were challenged with intravenous injection of 0.5x10^6 MICA expressing B16F10 melanoma cells. The mice were randomized into 3 cohorts with 4 mice in each. On days 1, 2, 4 and 6 after tumor challenge, the mice were injected (intraperitoneal route) with 100µl of end point sera from naïve, OVA-protein or MICA-ferritin vaccine immunized C57BL/6 mice. Mice were euthanized 14 days after tumor challenge; lungs were harvested and fixed in 10% neutral-buffered formalin and the number of pulmonary metastases was quantified. Mice injected with sera from MICA-ferritin vaccinated group (red square) had significantly fewer lung metastases compared to mice injected with sera from untreated, age matched control group (black round) and OVA-protein immunized group. **B**. sMICA level was lower in mice receiving sera from MICA-ferritin vaccinated group (red square) compared to mice receiving sera from naïve or OVA-protein immunized group.

What opportunities for training and professional development has the project provided?

The Wucherpfennig lab provided an inspiring environment to acquire cutting edge knowledge in the field of cancer immunology. The resources available in the lab such as Multitron Cell for large scale protein production, UPLC for assessing the quality of purified protein and BD LSRFortessa that can detect up to 18 color cell staining simultaneously helped in the advancement of this project. The progress of the project, experimental designs and specific strategies to address critical issues were discussed during weekly meetings with my mentor and joint monthly meetings with both my mentor and co-mentor. The excellent research environment at DFCI and Harvard Medical School (HMS) fostered my technical skills required for the completion of this project. In addition to acquiring all the essential technical skills, I attended the immunology seminar series at HMS where experts in the field of immunology and cancer research from around the globe were invited to present and discuss their on-going research. I also actively participated at weekly seminars, floor meetings and journal clubs. These seminars helped me to acquire an in-depth knowledge in the field of cancer research, critically analyze the work of other researchers, diversify my knowledge and helped me to develop innovative concepts which will be important for my future as an independent researcher in this rapid growing field of cancer research. I also had the opportunity to mentor 3 undergraduate summer students and a master's student during this funding period. This helped to strengthen my teaching and leadership skills. In addition to these, my participation at workshops and seminars for professional development hosted by Dana Faber post-doctoral office including manuscript writing, research ethics, lab management and grant-writing have provided me with the skills which are necessary to transition from training towards establishing an independent and successful academic career in this rapidly growing field of cancer research.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Melanomas are highly metastatic, and even patients in whom a small local lesion is successfully resected can later develop lethal metastases. Patients whose tumors express MICA and/or MICB could benefit from our vaccine. Our vaccine approach could help to prevent emergence of metastases in these patients which is the principal challenge in the treatment of melanoma. Recent clinical trials with monoclonal antibodies targeting inhibitory receptors such as CTLA-4 and PD-1 have shown significant survival benefits in in a subset of melanoma patients. This treatment approach requires repeated administration of recombinant antibodies and is very expensive.

Furthermore, these antibodies target key inhibitory feedback loops in the immune system designed to protect against autoimmunity. Significant autoimmune toxicity is a major limitation of these antibodies, in particular for CTLA-4 antibodies. Our vaccine is conceptually different from the existing vaccines as it prevents tumor escape from an important immune surveillance pathway. Endogenous polyclonal antibodies generated in response to MICA alpha3 vaccine could rapidly clear the immunosuppressive shed MICA/B from the patient sera and rescue NK cell function. In addition to this, polyclonal MICA/B alpha3 antibodies could inhibit shedding of these ligands for an extended period of time. Increased density of MICA/B makes tumor cells better targets for killing by both CD8 T cells and NK cells. CD8 T cells are critical effectors of tumor immunity, and NK cells are relevant for controlling tumor growth and preventing metastases. The MICA/B alpha3 vaccine creates immunological memory, and could thereby provide long term protection from tumor recurrence.
Current immunotherapies targeting tumor antigens or neo-antigens cause T cell exhaustion driven by chronic TCR stimulation due to lack of co-stimulatory molecules on tumor cells. Since MICA/B serve as co-stimulatory molecules for CD8 T cells, this vaccine can also be used in combination with other vaccine antigens to boost the efficacy of T-cell-dependent immunotherapy.

What was the impact on other disciplines?
The project helped in understanding and utilizing different biomaterials for testing the therapeutic application of the vaccine in treating melanoma tumors.

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
If the vaccine proves to be effective and safe in treating melanoma, it could be broadly utilized to treat and prevent many different cancer types, expressing MICA/B antigens. The low cost of the vaccine would allow it to be used in countries whose health care systems cannot afford highly expensive monoclonal antibody therapy. This vaccine could also be used in combination with other vaccines or other treatment approaches. From a conceptual perspective, this could be the first cancer vaccine that prevents escape from immune surveillance.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
The role of NKG2D pathway and the fc receptors could not be determined in the therapeutic effect of vaccine in subcutaneous model since breeding these mice took longer than expected. We are working towards dissecting this pathway in the subcutaneous B16F10 tumor model once we have the NKG2D knockout, Fcgr3 and Fcer1g knockout mice respectively.

The tumors growing out (subcutaneous B16F10-MICA) in the MICA vaccinated mice (**Figure 6A)** were found to be MICA negative. Upon further investigation, we realized that this was due to silencing of the lentiviral promoter driving MICA expression. We are working towards developing new MICA cell lines with CRISPR mediated knock-in of MICA into the B16F10 cells to prevent antigen loss due to lentiviral silencing.

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals.
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Nothing to report

  Journal publications.
  Nothing to report

  Books or other non-periodical, one-time publications.
  Nothing to report

  Other publications, conference papers, and presentations.
  Presentations –
  DFCI Cancer Immunology and Virology floor meeting – January 2017
  Wucherpfennig lab – monthly lab meetings
  Wyss Institute Immune Materials Platform Meeting – July 2017

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report

- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Soumya Badrinath
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	11
Contribution to Project:	Dr. Badrinath is responsible for the conception and design of all the studies. She executed, analyzed data, and presented the findings to the scientific community.
Funding Support:	None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.