Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Wistar Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/2013 – 06/30/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Russel E. Kaufman, M.D.

4. **Grant Contact Person's Telephone Number:** 215-898-3926

5. **Grant SAP Number:** 4100062226

6. **Project Number and Title of Research Project:** 2: Regulation of Immune Responses in Multiple Myeloma Bone Marrow Microenvironment

7. **Start and End Date of Research Project:** 01/01/2013 – 06/30/2014

8. **Name of Principal Investigator for the Research Project:** Yulia, Nefedova, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 405,498.27

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Nefedova, Yulia	Principal Investigator	55%	$118,742

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
CTL Immunospot Analyzer	This instrument is essential for performing ELISPOT assays, such as those shown in Figure 3 of this report, for measuring IFN-gamma secretion.	$48,210.00
LSRII-14/LSRII-18 Lasers	These lasers were added to existing flow cytometers to allow evaluation of surface marker expression on cells.	$59,378.04
-80 Freezer	Ultra-low temp freezer is required for storage of sensitive samples.	$12,302.73
Spectrophotometer	This spectrophotometer and plate reader is necessary to quantitate protein concentration and to conduct MTT assays.	$11,012.96
Lab Refrigerator	A lab-grade refrigerator is essential for storage of critical reagents and samples that must be kept cold but not frozen.	$3,985.42
Lab Freezer	A lab-grade freezer (-20C) is essential for storage of critical reagents and samples that must be frozen but that do not need ultra-low temp conditions.	$6,773.57
2 Centrifuges	Refrigerated and non-refrigerated bench top centrifuges are essential to tissue culture applications and for preparing samples for flow cytometry.	$13,794.67
Eppendorf PCR machine	PCR is essential for evaluating gene expression.	$12,402.17

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No X

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No X

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes X__ No_____

If yes, please describe your plans:

We are planning to apply for NIH funding (R01 mechanism) to continue this research.

12. Future of Research Project. What are the future plans for this research project?

We will employ strategies to inhibit myeloid-derived suppressor cells (MDSC) with the goal of inhibiting immune suppression and improving function of T cells in multiple myeloma.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No X

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No X

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No X

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No X

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No X

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No X

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Objectives

The goal of this project is to understand the function of bone marrow immature myeloid cells in multiple myeloma (MM), a blood cancer characterized by uncontrolled growth of malignant plasma cells preferentially in the bone marrow. Using *in vivo* immunocompetent mouse model of MM, we will investigate whether immature myeloid cells expand and accumulate in this disease. We will also evaluate whether these cells are functionally changed in MM. Specifically, the ability of immature myeloid cells to suppress immune responses, and thus become so-called myeloid derived suppressor cells (MDSC), will be determined. Our study will also provide an insight into the kinetic of antigen-specific immune response in MM and investigate the mechanisms by which MDSC could regulate function of immune cells in the bone marrow. Finally, we will determine whether targeting MDSC could improve anti-tumor immune responses leading to decrease MM burden.

The following Specific Aims will be addressed:

Specific Aim 1: Investigate the involvement of MDSC in regulation of immune responses in MM bone marrow (BM); and *Specific Aim 2:* Determine whether targeting MDSC would improve the anti-tumor immune response in MM. The knowledge gained from this project will be important, as it may justify a novel therapeutic approach for treatment of multiple myeloma based on targeting MDSC.

Progress report

To understand the role of myeloid-derived suppressor cells (MDSCs) in pathogenesis of multiple myeloma (MM), we utilized syngeneic mouse model of this disease. In this model, MM cell lines

(ATLN or DP42) injected intravenously into syngeneic mice are homing to the bone marrow (BM), and the tumor growth closely resembled human disease. Initially, we investigated whether MM tumor growth was accompanied by accumulation of MDSCs. In both ATLN and DP42 models, a significant increase in the proportion and absolute number of MDSCs in BM was observed as early as one week after tumor cell inoculation (Figure 1A and data not shown). From week two, the presence of MDSCs in BM was gradually decreased; and, three weeks after tumor cell inoculation, the proportion of MDSCs in BM was significantly reduced as MM cells expanded and substituted all hematopoietic cells in BM. In spleen, the proportion and absolute number of MDSCs was also significantly increased one week after tumor cell inoculation. However, this cell population continued to grow during week two post tumor injection. Only at the end of week three, when mice became moribund and MM cells expanded, the presence of MDSCs in spleens declined (Figure 1B). We next investigated whether MDSCs isolated from BM of MM-bearing animals acquired an ability to suppress antigen-specific T cell responses during tumor growth. Gr-1$^+$CD11b$^+$ MDSCs were isolated from BM of MM ATLN-bearing mice one or two weeks after the injection of tumor cells by flow sorting using Aria instrument (BD). As a control, Gr-1$^+$CD11b$^+$ immature myeloid cells (IMC) isolated from BM of tumor-free mice were used. The ability of MDSC and IMC to suppress antigen-specific T cell response was tested in ELISPOT and proliferation assays. MDSC and IMC were added to Pmel-1 transgenic splenocytes (responders) in the presence of specific gp100 (EGSRNQDWL) or control peptides. IFN-γ production evaluated by ELISPOT assay and proliferation by [^{3}H]-thymidine incorporation were determined after 48 hours and 96 hours, respectively. MDSC demonstrated potent suppressive activity, whereas IMC did not inhibit T-cell responses (Figure 1C,D). Taken together, our data demonstrated that immature myeloid cells possessing immunosuppressive activity accumulated in MM tumor-bearing animals.

To confirm the relevance of our *in vivo* data we evaluated the presence of MDSCs defined as CD11b$^+$CD14$^-$CD33$^+$ cells in the BM and peripheral blood (PB) of newly diagnosed, non-treated patients with multiple myeloma (MM). Cells with the same phenotypes in control donors were called immature myeloid cells (IMC). Mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation, labeled with corresponding antibodies and subjected to flow cytometry using LSR II instrument (BD). Our data demonstrated a significant ($p<0.05$) accumulation of MDSC in BM of MM patients, as compared to healthy donors (Figure 2). MDSC represented 41.1±5.3% (range from 13.3% to 75.9%) of BM cells in MM, while IMC with the same phenotype only 22.9±2.8% (range from 7.7% to 33.3%). A significant accumulation of MDSC was also observed in PB of MM patients (Figure 2). To verify whether the population of cells with the phenotype of MDSC that accumulates in BM of MM patients could be functionally defined as MDSC, we determined their immune suppressive activity. Populations of MDSC or IMC were isolated by flow sorting using FASCAria instrument (BD). Human T cells were purified from PB mononuclear cells (PBMC) obtained from healthy donors using T cell enrichment columns (R&D systems). Dendritic cells were generated *in vitro* from PBMC obtained from a different donor. T cells ($1x10^5$) were stimulated with lipopolysaccharides (LPS)-matured dendritic cells ($1.5x10^4$), with or without MDSC present in the co-culture. The number of IFN-γ-producing T cells was evaluated in an ELISPOT assay on automatic counter. Proliferation of T cells was measured by ^{3}H-Thymidine incorporation. IMC isolated from BM of healthy donors were used as control. A significant inhibition of T-cell activity by MDSC was

observed (Figure 3A,C), whereas IMC lacked suppressive activity (Figure 3B,D). These data indicate that MDSC with immune suppressive activity accumulated in BM of MM patients.

We evaluated an expression of several genes previously implicated in MDSC-mediated immune suppression. Gene expression of arginase-1, Cybb and Cox-2 was significantly up-regulated in MDSC isolated from BM of MM patients as compared to IMC isolated from BM of healthy donors (Figure 4A). There was no difference in ROS and iNOS level between MDSC and IMC (Figure 4B-D).

To understand the role of myeloid-derived suppressor cells (MDSC) in pathogenesis of multiple myeloma (MM), we utilized syngeneic mouse model of this disease. In this model, MM cell lines ATLN and DP42 derived from double transgenic bcl-xL/c-myc mice were injected i.v. into syngeneic mice. As MM cells were homing to the bone marrow (BM), the tumor growth in this model closely resembled human disease. To address a possible contribution of myeloid cells in progression of MM, we depleted these cells *in vivo* using CD11b-diphtheria toxin receptor (DTR) mouse model. In this model, BM of wild type mice was reconstituted with BM cells obtained from CD11b-DTR mice. Administration of diphtheria toxin (DT) resulted in approximately 3 fold reduction in absolute number of myeloid cells in BM. Depletion of CD11b+ myeloid cells led to significantly improved survival of mice from MM (Figure 5).
To further evaluate a possible role of MDSC in MM, we utilized S100A9KO mice. Under physiological conditions, these mice demonstrated normal myeloid cell differentiation. However, when challenged with tumor cells, S100A9KO mice showed a reduced accumulation of MDSC as compared to their wild-type (WT) counterparts. One of the major effects of MDSC is the inhibition of antigen-specific immune responses. Rapid growth of MM in BCM and DP42 models, 100% tumor take, and the lack of spontaneous rejection suggested that these tumor cells are likely to be poorly immunogeneic.

To investigate the effect of MDSC in the model of a more immunogeneic tumor, we generated a DP42 cell line with a stable overexpression of OVA – DP42-OVA. DP42-OVA cells had similar kinetic of growth *in vitro* as compared to parental DP42 cells (data not shown). However, S100A9KO mice inoculated with DP42-OVA cells demonstrated a significantly improved survival (p=0.009) (Figure 6A) and a delayed tumor growth in BM (Figure 6B), as compared to WT mice. We compared the presence of Gr-1$^+$CD11b$^+$ MDSC in BM of WT and S100A9 mice one week after MM inoculation. At this point, the number of tumor cells in BM (Figure 6B) and spleens (Figure 6C) of both groups of mice was the same. MDSC in BM of DP42-OVA-bearing WT mice was increased, as compared to tumor-free mice. The presence of MDSC in BM of DP42-OVA-bearing S100A9KO mice was significantly (p=0.04) lower (Figure 6D). MDSC isolated from BM of DP42-OVA-bearing WT mice one week after tumor inoculation suppressed antigen-specific T-cell response, whereas MDSC from S100A9KO MM-bearing mice lacked this ability (Figure 6E). These results suggest that the loss of immune suppressive activity in S100A9KO mice was likely the result of a decreased proportion of immune suppressive MDSC among BM Gr-1$^+$CD11b$^+$ cells.

To investigate the involvement of the immune mechanisms in decreased tumor growth of MM in S100A9KO mice, we depleted CD8$^+$ T cell using CD8 monoclonal antibody (Ab, Clone: 53-6.72, BioXCell, West Lebanon, NH). Mice were treated i.p. with 200µg of CD8 antibody or

control IgG2b (clone LTF-2, BioXCell) every four days, beginning four days after tumor cell injection (total of six injections of anti-CD8 Ab or control IgG). Administration of this antibody completely abrogated the improved survival of S100A9KO mice inoculated with DP42-OVA MM cells (Figure 7A). To investigate whether the decreased presence of MDSC in MM-bearing S100A9KO mice would result in an improved tumor-specific immune response, we measured the presence of OVA-specific $CD8^+$ T cells in DP42-OVA-bearing mice using SIINFEIKL-$H2K^b$ pentamers. After one week, S100A9KO mice had a higher presence of pentamer-positive $CD8^+$ T cells in BM; however, the difference was not statistically significant. By week three, however, the presence of pentamer-positive $CD8^+$ T cells was significantly higher in BM of DP42-OVA-bearing S100A9KO than in WT mice (Figure 7B). Similar kinetic was observed in spleens of these mice (Figure 7C). However, at all time points the proportion of pentamer-positive $CD8^+$ T cells in spleens was more than 10-fold lower than in BM (Figure 7D, E), indicating that BM was the primary site of accumulation of antigen-specific $CD8^+$ T cells in S100A9KO MM-bearing mice. We also evaluated the presence of IFN-γ^+ (Th1-type) $CD4^+$ T cells in MM-bearing mice. A slight increase in proportion of these cells was observed one week after tumor inoculation in BM of S100A9KO mice. A week later the differences became significant (Figure 7D). There was no increase in the proportion of IFN-γ positive cells in spleens (Figure 7E). To confirm the contribution of MDSC in MM tumor growth, an adoptive transfer of $Gr1^+CD11b^+$ cells isolated from BM of WT DP42-bearing mice was performed into recipient S100A9KO DP42-OVA bearing mice. $Gr1^+CD11b^+$ MDSC were isolated from BM of DP42-bearing mice by flow sorting ten days after tumor cell inoculation. MDSC (5×10^6) were injected i.v. into tail vein of DP42-OVA bearing mice every four days, beginning on day four after DP42-OVA tumor cell injection. The mice received a total of four injections of MDSC. Transfer of WT MDSC into MM-bearing S100A9KO mice resulted in significantly decreased survival of these mice (Figure 7F). Thus, using a mouse model of MM and knockout mice with defective MDSC accumulation, we showed that MDSC could be directly responsible for the tumor-specific immune suppression observed in BM in this disease and demonstrated that MDSC can be considered as a possible therapeutic target in this disease.

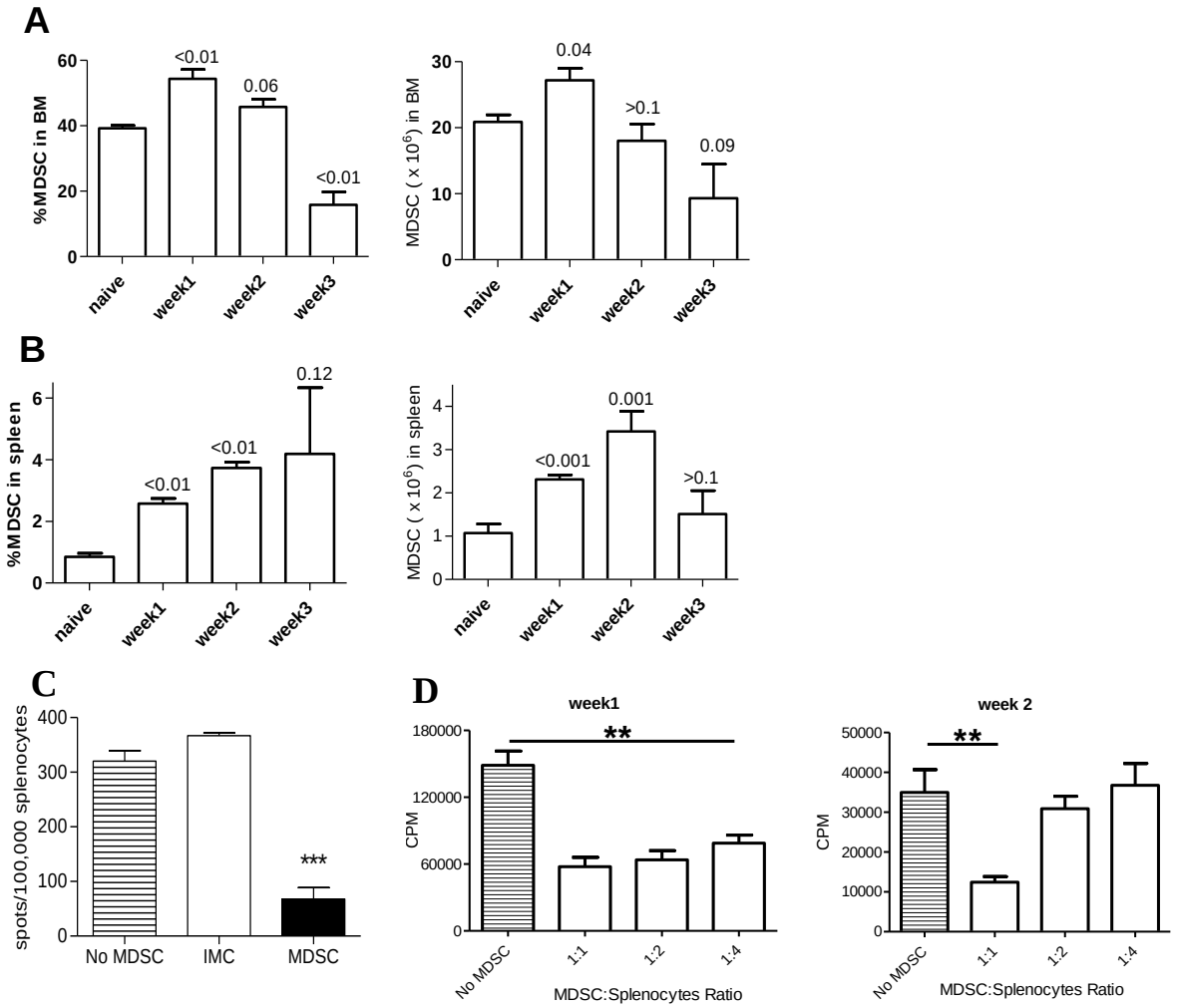

Figure 1. ***Accumulation and function of MDSC in MM-bearing mice.*** MM tumors were established by i.v. tail vein injection of 10^5 ATLN cells into syngeneic mice. Mice were euthanized at indicated time points after tumor cell inoculation (3-4 mice per time point). As a control (naïve), tumor-free mice were used. Proportion (left panels) and absolute number (right panels) of $Gr1^+CD11b^+$ cells in (**A**) BM and (**B**) spleen are shown. (**C,D**) $Gr1^+CD11b^+$ cells isolated from BM of ATLN-bearing or naïve mice were cultured with splenocytes from Pmel-1 transgenic mice in the presence of control or specific peptides. Each condition was set up in triplicates. (**C**) MDSCs or IMCs were mixed with splenocytes in 1:1 ratio. INF-γ production by T cells was measured in ELISPOT assay. Shown are the numbers of spots calculated by subtracting background values (cells stimulated with control peptides) from specific values (cells stimulated with specific peptide). Three mice per group were used. *** - statistically significant difference between IMC and MDSC (p=0.000073). (**D**) Proliferation assay. Results are mean values obtained for 3 mice with each condition set up in triplicates. Proliferation was measured by ^{3}H-Thymidine incorporation. ** - statistically significant difference (p<0.001).

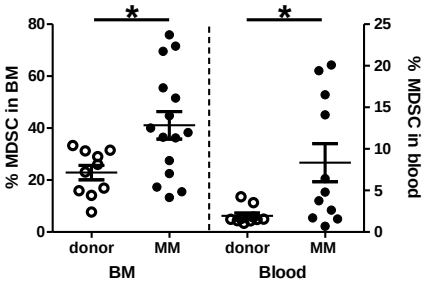

Figure 2. ***Accumulation of MDSC in patients with MM.*** BM and PB samples were obtained from patients with MM (n=15 and n=11, respectively) or healthy donors (n=6 and n=10, respectively). Mononuclear cells were labeled with specified antibodies and analyzed by flow cytometry. Proportion of $CD11b^{+}CD14^{-}CD33^{+}$ MDSC was determined. Presented data show proportion of these cells calculated among $CD138^{-}$ non-myeloma cell. * - statistical significance between groups ($p<0.05$).

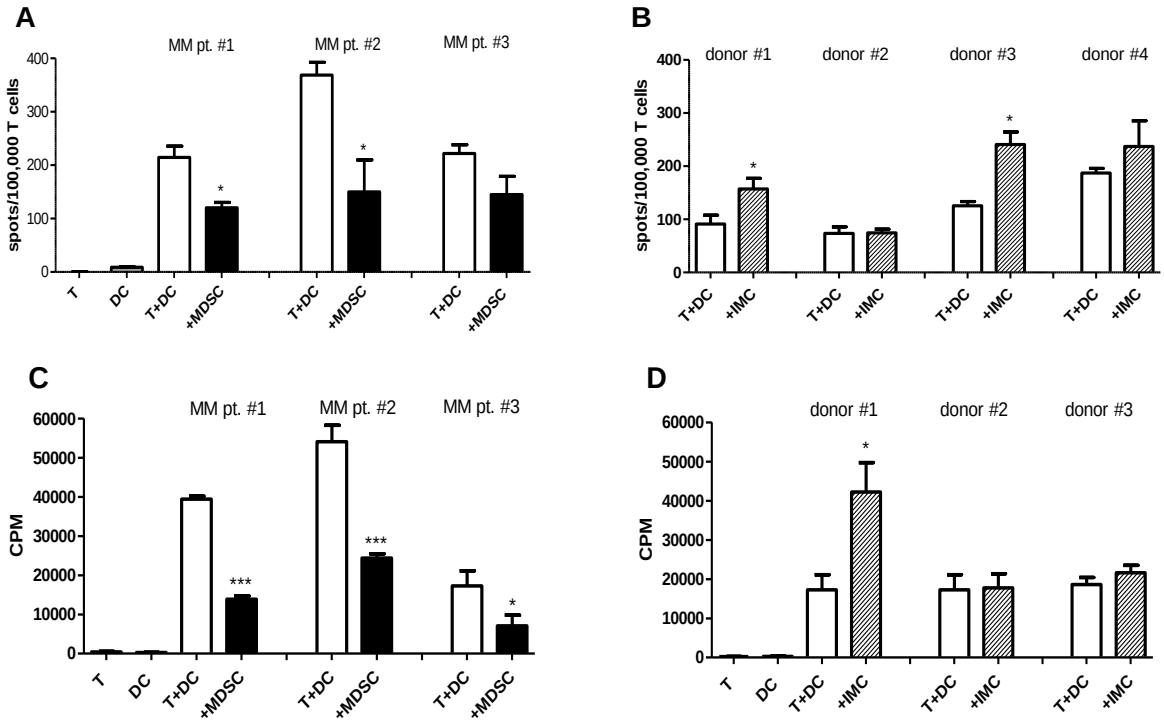

Figure 3. ***Suppressive activity of MDSC isolated from patients with MM.*** $CD11b^{+}CD14^{-}CD33^{+}$ cells were isolated by sorting of BM from MM patients (**A,C**) or healthy donors **(B,D).** Sorted cells were added at 1:1 ratio to purified T cells stimulated by allogeneic dendritic cells. (**A,B**) The secretion of IFN-γ was measured by ELISPOT after 48 hours of incubation. (**C-D**) T cell proliferation was measured by ^{3}H-Thymidine incorporation. Each condition was set up in triplicate. Statistically significant differences between samples with and without MDSC: * - $p<0.05$; *** - $p<0.005$.

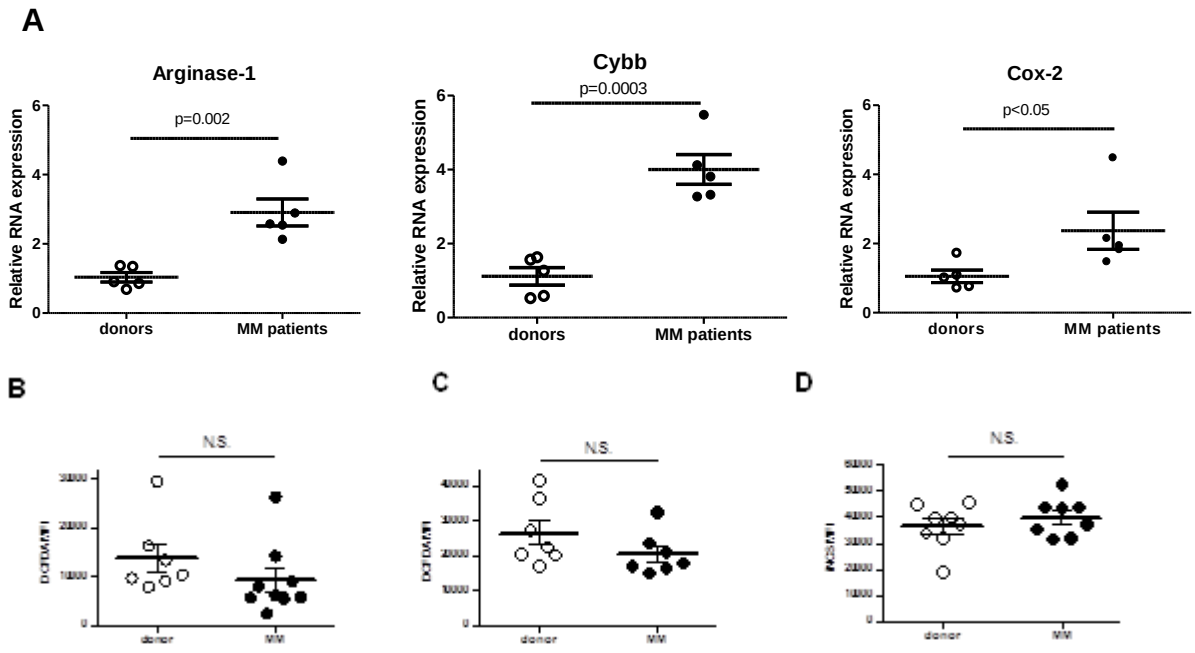

Figure 4. ***Factors involved in MDSC suppression activity in patients with MM.*** **(A)** $CD11b^{+}CD14^{-}CD33^{+}$ cells were isolated from BM of newly diagnosed patients with MM (n=5) or healthy donors (n=5) by sorting using FACSAria instrument. Expression of indicated genes was determined by qPCR and normalized to the expression of housekeeping gene (actin). Statistically significant differences between MM and donor groups are indicated. (**B-D**) BM samples were obtained from patients with MM (n=8) or healthy donors (n=7). Mononuclear cells were labeled with DCFDA dye and incubated without stimulation (**B**) or stimulated with PMA (**C**) followed by surface staining with antibodies against CD33, CD11b, and CD14. DCFDA MFI was determined by flow cytometry in $CD11b^{+}CD14^{-}CD33^{+}$ cells. (**D**) iNOS MFI was determined by flow cytometry in gated $CD11b^{+}CD14^{-}CD33^{+}$ population of cells.

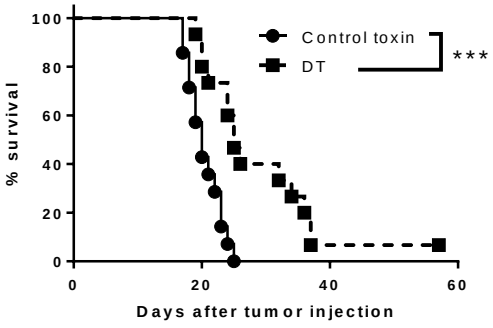

Figure 5. ***Depletion of myeloid cells results in improved survival of mice from MM.*** BM of wild type mice was reconstituted with BM cells isolated from CD11b-DTR mice. Mice were injected with DT in order to deplete CD11b+ cells or control toxin followed by inoculation of DP42 MM cells. Survival of MM tumor-bearing CD11b-DTR BM chimeric mice was evaluated. *** - p= 0.0003.

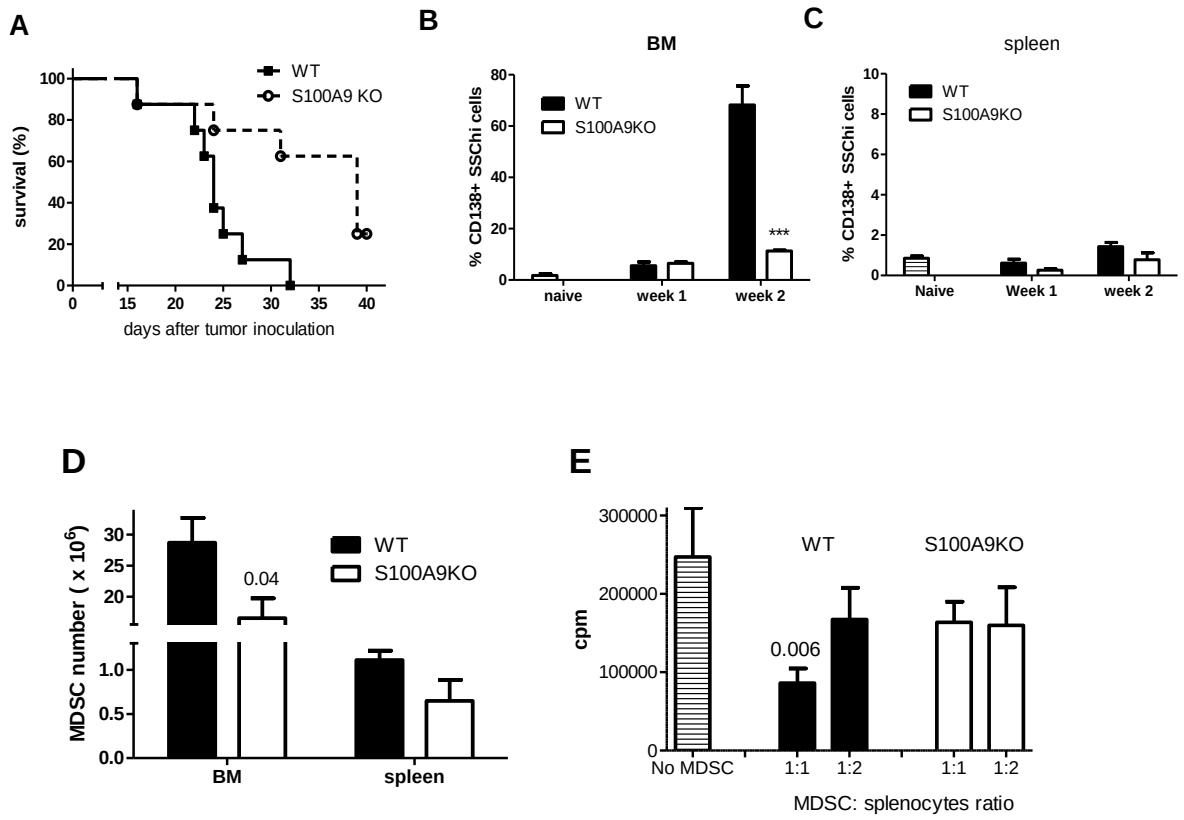

Figure 6. Growth of immunogenic MM tumors in S100A9KO mice. (**A-D**) DP42-OVA MM tumors were established in S100A9KO or WT mice. (**A**) Survival of S100A9KO (n=6) and WT (n=8) mice was evaluated. Log rank analysis was used to determine statistical significance between groups (p=0.009). (**B,C**) S100A9KO or WT DP42-OVA-bearing mice were euthanized at indicated times (3 mice per time point) and tumor burden in BM **(B)** and spleen **(C)** was determined by measuring the proportion of $CD138^{+}SSC^{hi}$ MM cells. *** - indicates statistically significant difference with $p<0.001$. (**D**) Absolute number of $Gr1^{+}CD11b^{+}$ cells was determined in DP42-OVA S100A9KO and WT mice one week after MM cell inoculation. Three mice per group were analyzed. (**E**) Sorted BM $Gr1^{+}CD11b^{+}$ MDSCs from DP42-OVA-bearing S100A9KO or WT mice one week after tumor cell inoculation were mixed with Pmel-1 splenocytes at indicated ratio in the presence of specific or control peptides. Proliferation was measured using ^{3}H-Thymidine incorporation. Statistically significant difference between no MDSC and WT MDSC groups is shown.

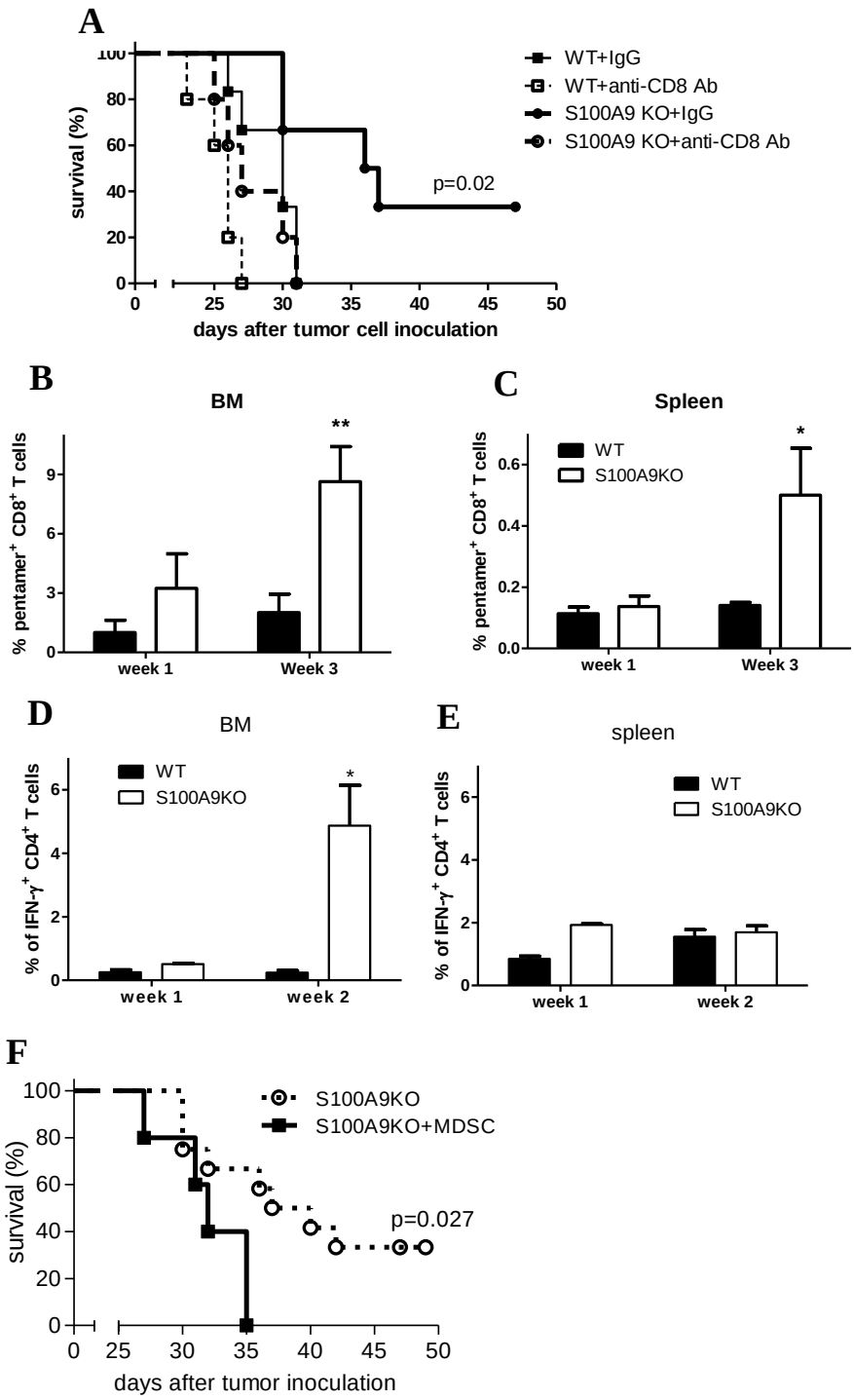

Figure 7. Tumor-specific T cell responses in MM. DP42-OVA tumors were established in S100A9KO or WT mice. (**A**) Four days after tumor cell inoculation each group was split into two and treatment with anti-CD8 antibody or control IgG was initiated. Mice survival was evaluated. (**C-D**) DP42-OVA bearing mice were euthanized at indicated time points. BM and spleens were collected and labeled with anti-CD8 antibody and SIINFEKL-H2-K^b pentamer

conjugated with FITC and analyzed by flow cytometry. (**B**) Proportion of ova-specific $CD8^+$ T cells in BM and spleens (**C**) * - statistical significance $p<0.05$; ** - statistical significance $p<0.01$ between S100A9KO and WT group of mice. BM **(D)** and spleens **(E)** were collected from DP42-OVA bearing S100A9KO and WT mice. Proportion of IFN-γ^+CD4^+ T cells was determined by flow cytometry. Three mice per group were analyzed. All experiments were repeated twice. * - indicate significant difference $p<0.05$. (**F**) MDSC were isolated from DP42-bearing WT mice and $5x10^6$ cells were injected i.v. into DP42-OVA-bearing S100A9KO mice on days 4, 8, 12 and 16 after tumor cell inoculation. Survival of DP42-OVA bearing S100A9KO mice with (n=5) or without (n=12) transferred MDSC was determined.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
X_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
X_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes X___ No_____

If yes, please describe your plans:

We anticipate submitting a manuscript in November 2014.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No_X_

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No X

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME **NEFEDOVA, Yulia**	POSITION TITLE **Assistant Professor**
eRA COMMONS USER NAME **NEFEDOVAY**	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
State Medical University, St-Petersburg, Russia	M.D.	06/93	Medicine
State Medical University, StPetersburg, Russia	Ph.D.	10/99	Molecular Genetics

A. PERSONAL STATEMENT

During the last 14 years, my research has been focusing on the role of tumor microenvironment in regulation of tumor progression and chemoresistance. Specifically, I was interested in investigating the contribution of different bone marrow cell populations in growth and chemoresistance of multiple myeloma cells and molecular mechanisms responsible for that. Recently, my lab has demonstrated an involvement of bone marrow myeloid-derived suppressor cells in multiple myeloma progression. We are currently trying to identify molecular mechanisms responsible for this effect and develop approaches to target these molecules/pathways. The current application builds logically on my prior work and is focused on the role played by S100A9 protein produced by myeloid-derived suppressor cells in myeloma progression. I have a background in medicine, completed residency in hematology, postdoctoral training in cancer cell biology and signaling, and have several years of independent experience as a principal investigator funded by R01 award from NIH/NCI and grants from private foundations. During all these years I have acquired the necessary expertise and experience making me well qualified to further develop the research outlined in the proposed specific aims.

B. POSITIONS AND HONORS

Positions and Employment

09/1993-08/1994	Internship in Laboratory Medicine, State Medical University, St. Petersburg, Russia
09/1994-08/1996	Residency in Hematology, State Medical University, St. Petersburg, Russia
05/2007-12/2007	Research Assistant Professor, H. Lee Moffitt Cancer Center, Tampa, FL
01/2008-04/2013	Assistant Member, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL
01/2008-04/2013	Assistant Professor, University of South Florida, Tampa, FL
05/2013-present	Assistant Professor, The Wistar Institute, Philadelphia, PA

Professional Memberships

2006-present	Member, American Society of Hematology
2009-present	Member, American Association for Cancer Research

Honors and Awards

2002	Multiple Myeloma Research Foundation Research Fellow Award
2004-2007	Ruth L. Kirschstein National Research Service Award
2009	Miles for Moffitt Milestone Award

C. SELECTED PEER-REVIEWED PUBLICATIONS (Selected from 23 publications)

1. Plescia J, Salz W, Xia F, Pennati M, Zaffaroni N, Diadone MG, Meli M, Dohi T, Fortugno P, Nefedova Y, Gabrilovich DI, Colombo G, Altieri DC. (2005) Rational design of shepherdin, a novel anticancer agent. ***Cancer Cell*** 7(5):457-468. PMID:15894266.
2. Nefedova Y, Cheng P, Gilkes D, Blaskovich M, Beg AA, Sebti SM, Gabrilovich DI. (2005) Activation of dendritic cells via inhibition of Jak2/STAT3 signaling. ***J Immunol***. 175(7):4338-4346. PMCID: PMC1351251.
3. Nefedova Y, Nagaraj S, Rosenbauer A, Muro-Cacho C, Sebti SM, Gabrilovich DI. (2005) Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. ***Cancer Res***. 65(20):9525-9535. PMCID: PMC1351362.
4. Cheng P, Nefedova Y, Corzo CA, Gabrilovich DI. (2007) Regulation of dendritic cell differentiation by bone marrow stroma via different Notch ligands. ***Blood*** 109(2):507-515. PMCID: PMC1766374.
5. Nefedova Y, Fishman M, Sherman S, Wang X, Beg AA, Gabrilovich DI. (2007) Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. ***Cancer Res*** 67(22):11021-11028. PMID:18006848.
6. Nefedova Y, Sullivan DM, Bolick SC, Dalton WS, Gabrilovich DI. (2008) Inhibition of Notch signaling induces apoptosis of myeloma cells and enhances sensitivity to chemotherapy. ***Blood*** 111(4):2220-2229. PMID:18039953.
7. Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, Corzo A, Cho HI, Celis E, Lennox B, Knight SC, Padhya T, McCaffrey TV, McCaffrey JC, Antonia S, Fishman M, Ferris RL, Kagan VE, Gabrilovich DI. (2010) Lipid accumulation and dendritic cell dysfunction in cancer. ***Nat Med***. 6(8):880-886. PMID: 20622859. PMCID: PMC2917488.
8. Li M, Chen F, Clifton N, Sullivan DM, Dalton WS, Gabrilovich DI, Nefedova Y. (2010) Combined inhibition of Notch signaling and Bcl-2/Bcl-xL results in synergistic anti-myeloma effect. ***Mol Cancer Ther***. 9(12):3200-3209. PMCID: PMC3058809.
9. Chen F, Pisklakova A, Li M, Baz R, Sullivan DM, Nefedova Y. (2011) Gamma-secretase inhibitor enhances the cytotoxic effect of bortezomib in multiple myeloma. ***Cell Oncol***. 34(6):545-551. PMCID: PMC3417352.
10. Youn JI, Kumar V, Collazo M, Nefedova Y, Condamine T, Cheng P, Villagra A, Antonia S, McCaffrey JC, Fishman M, Sarnaik A, Horna P, Sotomayor E, Gabrilovich DI. (2013) Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer. ***Nat Immunol***. 4(3):211-220. PMCID: PMC3578019.
11. Liu H, Zhou J, Cheng P, Ramachandran I, Nefedova Y, Gabrilovich DI. (2013) Regulation of dendritic cell differentiation in bone marrow during emergency myelopoiesis. ***J Immunol***. 191(4):1916-1926. PMCID: PMC3787311.
12. Ramachandran IR, Martner A, Pisklakova A, Condamine T, Chase T, Vogl T, Roth J, Gabrilovich D, Nefedova Y. (2013) Myeloid derived suppressor cells regulate growth of multiple myeloma by inhibiting T cells in bone marrow. ***J Immunol***. 190(7):3815-3823. PMCID: PMC3608837.