AWARD NUMBER:
W81XWH-16-1-0207

TITLE:
Myeloid-Derived Suppressor Cells in Checkpoint Protein Inhibition for Melanoma

PRINCIPAL INVESTIGATOR:
Jeffrey Weber

RECIPIENT:
New York University
New York NY 10016

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Fort Detrick, Maryland 21702-5012

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Distribution is unlimited

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New York University
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New York, NY 10016-6402

8. PERFORMING ORGANIZATION REPORT NUMBER

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Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Myeloid-derived suppressor cells (MDSC) are one of the major negative regulators of immune responses in cancer closely associated with negative outcome of PD1 therapy in metastatic melanoma. TRAIL-R DR5 is selectively up- regulated on MDSC. The goal of this study is to test the hypothesis that agonistic TRAIL-DR5 antibody DS-8273a will be well tolerated and augment the clinical efficacy of PD-1 blocking antibody nivolumab by impacting on MDSC. DS-8372a at low doses (4 and 8 mg/kg) was well tolerated with 2 excellent responses in 6 patients and one mixed response; it did not affect populations of MDSC or other myeloid and lymphoid cells, but monocytic MDSC function was augmented. In the first 4 patients we evaluated the response of T cells to melanoma derived pool of overlapping peptides in IFN-γ ELISPOT assay. In one patient we observed substantial increase in the response to peptides after 3 cycles of treatment. These results are preliminary. Moreover, the dose of antibody was very low to expect substantial responses. We anticipate that next two doses (16 mg/kg and 24 mg/kg) with escalation occurring early in October will provide more clear data.

15. SUBJECT TERMS
Myeloid derived suppressor cells, TNR-related apoptosis induced ligand-receptors

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
		U			USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	29	19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Myeloid-derived suppressor cells (MDSC) are one of the major negative regulators of immune responses in cancer. Increased presence of MDSC is closely associated with negative outcome of PD1 therapy in metastatic melanoma. Previous findings from our laboratory identified TRAIL-R DR5 as selectively up-regulated on MDSC. In phase I trial agonistic DR5 antibody DS-8273a selectively depleted MDSC without affecting neutrophils and monocytes. The goal of this study is to test the hypothesis agonistic TRAIL-DR5 antibody DS-8273a will be well tolerated and augment the clinical efficacy of PD-1 blocking antibody nivolumab by impacting on MDSC. We plan to evaluate the immunoregulatory activity of DS-8273a administered in combination with nivolumab in subjects with unresectable Stage III or Stage IV melanoma, and to explore the mechanisms by which the TRAIL-DR5 agonistic antibody depletes MDSC.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

myeloid derived suppressor cells
polymorphonuclear leukocytes
inducible nitric oxide synthetase
transforming growth factor beta
major histocompatibility complex
reactive oxygen species
peroxynitrate
quantitative reverse transcription polymerase chain reaction
endoplasmic reticulum
inositol-requiring enzyme 1
x-box binding protein-1
osteoprotegerin
TNF-related apoptosis induced ligand-receptors
cytotoxic T lymphocyte antigen-4
programmed death 1
monoclonal antibody
overall survival
progression-free survival
matrix-assisted, laser desorption ionization time-of-flight
protein disulfide isomerase

3. **ACCOMPLISHMENTS:** The 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.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

In this DOD Team Science proposal, approximately 38 patients will be treated on a clinical phase I dose escalation trial of antibody DS-8372a combined with nivolumab followed by a 20 patient expansion cohort at the recommended phase II dose of DS-8372a. Blood, tumor and sera will be obtained from all patients on the trial for the performance of correlative and mechanistic assays designed to promote our understanding of the pathways of resistance to treatment with PD-1 blockade. All patients will be treated at NYU Medical Center in New York City, and the correlative assays will be performed at NYU, the Wistar Institute in Philadelphia,
PA and at Biodesix, in Boulder, CO.

Approximately 30 pre-treatment PBMC specimens obtained by leukapheresis, 30 tumor biopsies, and 36 sets of sera, assuming a 20% drop out rate for pheresis and/or biopsies will be obtained. Approximately 90 post-treatment PBMC specimens (30 obtained by leukapheresis at time 12 weeks, peripheral blood draws in green top tubes at time 2, 3, 6, 9, 12, and 24 weeks), 30 tumor biopsies at week 12, and 108 sets of sera (weeks 5, 12 and 24), assuming a 20% drop out rate for pheresis and/or biopsies will be obtained.

Over the three months before the review of the current proposal, the protocol that this proposal is based on will be submitted to the peer review committee of the Perlmutter Cancer Center at NYU, which will take one months, and then 2 months will be required for the review by the Institutional Review Committees at NYU Medical Center and the Wistar Institute.

It is anticipated that 3 additional months will be required for all regulatory review and scrutiny. They would include:

c. 3 months for regulatory review and approval by the USAMRMC Human Research Protection Office (HRPO).

d. An IND application will be filed for the performance of the protocol of this proposal, and it will occur simultaneously with the regulatory review and approval by the USAMRMC HRPO.

Based on the timelines above, accrual to the trial that this proposal is based around would begin approximately 3 months after funding is initiated.

Accrual would proceed for 2 years then an additional 9 months would be required for final performance of the assays of this proposal in aims 1, 2 and 3, totaling 3 years in all.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

In summary, we treated the first 6 patients in the first two cohorts without significant toxicity on the trial; observed two excellent responses and one mixed response so far; there have been no dose limiting toxicities thus far. All toxicities have been of grades 1 and 2. The protocol has been amended to correct some minor errors, and to adjust the dose limiting toxicity period to 6 instead of 12 weeks per current custom for immunotherapy trials. The FDA had no issues with this. The data safety and monitoring committee (DSMC) met to evaluate the trial on 8-24-17, and had no major issues with the trial. Their report is attached. Dose escalation will continue to 16 mg/kg of DS 8273a in 4 weeks. Leukephereses and biopsies as well as all other blood draws have been carried out successfully. We are carrying out phenotypic analyses on the first three PBMC samples currently, using a new flow cytometer that measures 28 color samples. Progress by coinvestigators Drs. Hu and Gabrilovich are noted below.

1) major activities;

- We have obtained all regulatory approvals;
- We have obtained 34 samples of blood from 6 patients that were treated with 2 (low) doses of DS-8372a: 4 mg/kg and 8 mg/kg. Samples were processed and evaluated.

2) specific objectives;

To determine whether DS-8372a at different doses can selectively eliminated MDSC in patients. To evaluate possible clinical impact of DS-8372a on clinical response of patients treated with nivolumab.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative);

During first funding period we have obtained samples from 6 patients treated with lowest doses of DS-8372a : 4 mg/kg and 8 mg/kg. Samples were obtained at different time points during the treatment. Only few patients completed the entire treatment schedule. The results of the analysis are described below.

We evaluated frequency and absolute number of populations of MDSC: early stage MDSC (eMDSC), PMN-MDSC and M-MDSC, as well as monocytes, dendritic cells and different populations of lymphocytes (CD4+, CD8+, NK cells). In previous studies we observed the effect of DS-8372a antibody at dose 24 mg/kg. Low doses were selected to ascertain lack of toxicity. So, we did not expect any effect of DS-8372a at doses 4 and 8 mg/kg. Surprisingly there was a trend to decrease in the population of eMDSC in patients **(Fig. 1, 2)**. Because of small number of patients we could not separate them based on pre-treatment level of MDSC. No other populations were decreased. In contrast increase of monocytes was observed. The number of patients studied was too small to make any conclusion. We did not detect any changes in the expression of TRAILR in M-MDSC or PMN-MDSC (**Fig. 3**). Evaluation of two cohort of patients (4 mg/kg and 8 mg/kg) did not demonstrate any differences (**Fig. 4-6**). In 4 patients we evaluated response of T cells to melanoma derived pool of overlapping peptides (JPT pep mix) in IFN-γ ELISPOT assay. In all four patients one cycle of treatment did not result in changes in response. However, in one patient we observed substantial increase in the response to peptides after 3 cycles of treatment **(Fig. 7)**.

All these results are too preliminary to make a conclusion. Moreover, the dose of antibody was very low to expect substantial response. We anticipate that next two doses (16 mg/kg and 24 mg/kg) will provide more clear data of the response.

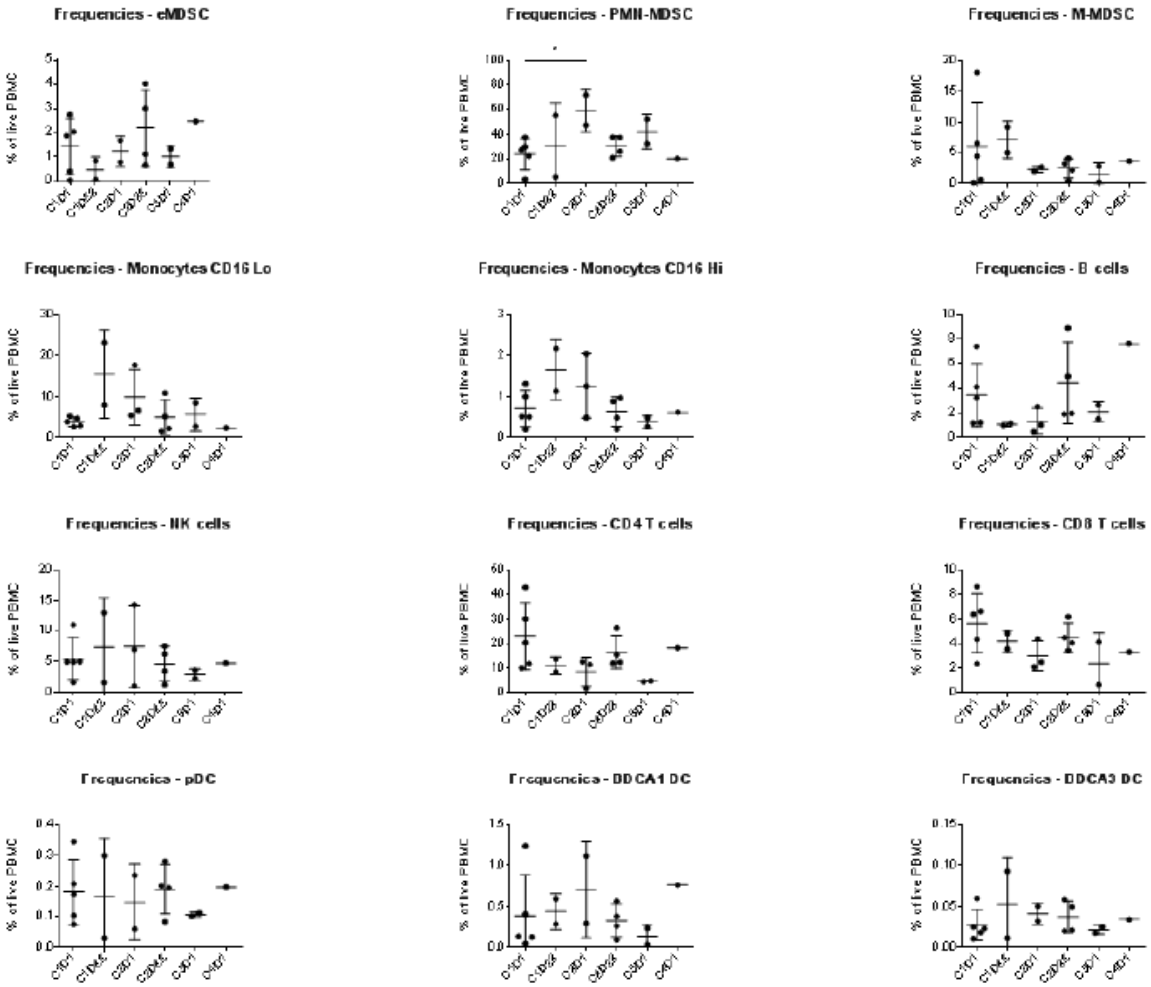

Figure 1. Frequency of myeloid and lymphoid population in peripheral blood of patients during the treatment. Results of individual patients are shown.

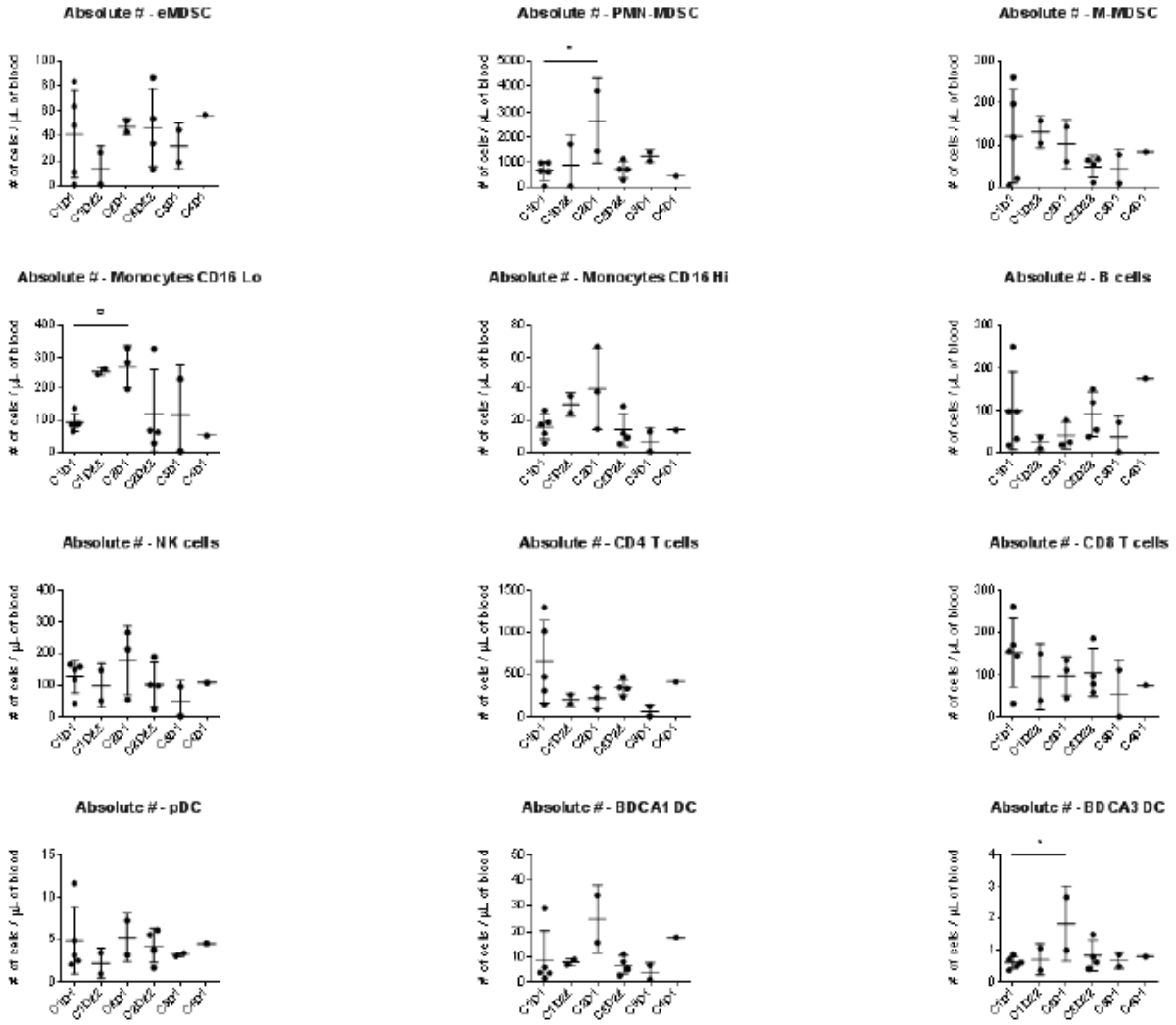

Figure 2. Absolute number of myeloid and lymphoid population in peripheral blood of patients during the treatment. Results of individual patients are shown.

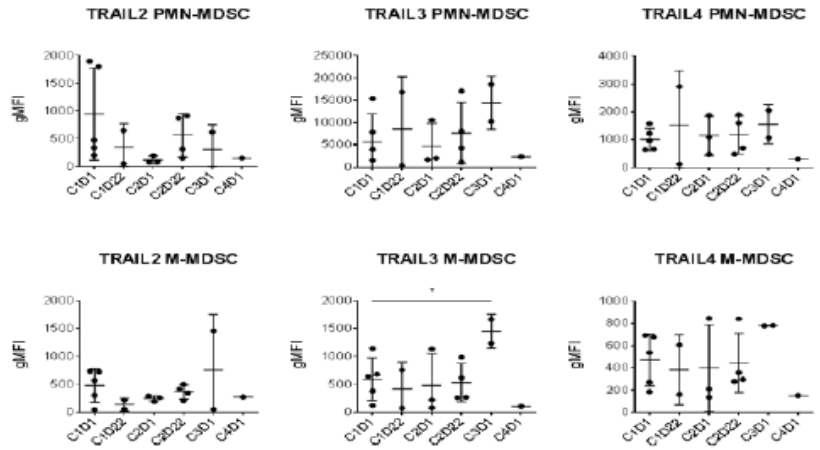

Figure 3. Expression of TRAIL receptors on PMN-MDSC and M-MDSC. Results of individual patients are shown.

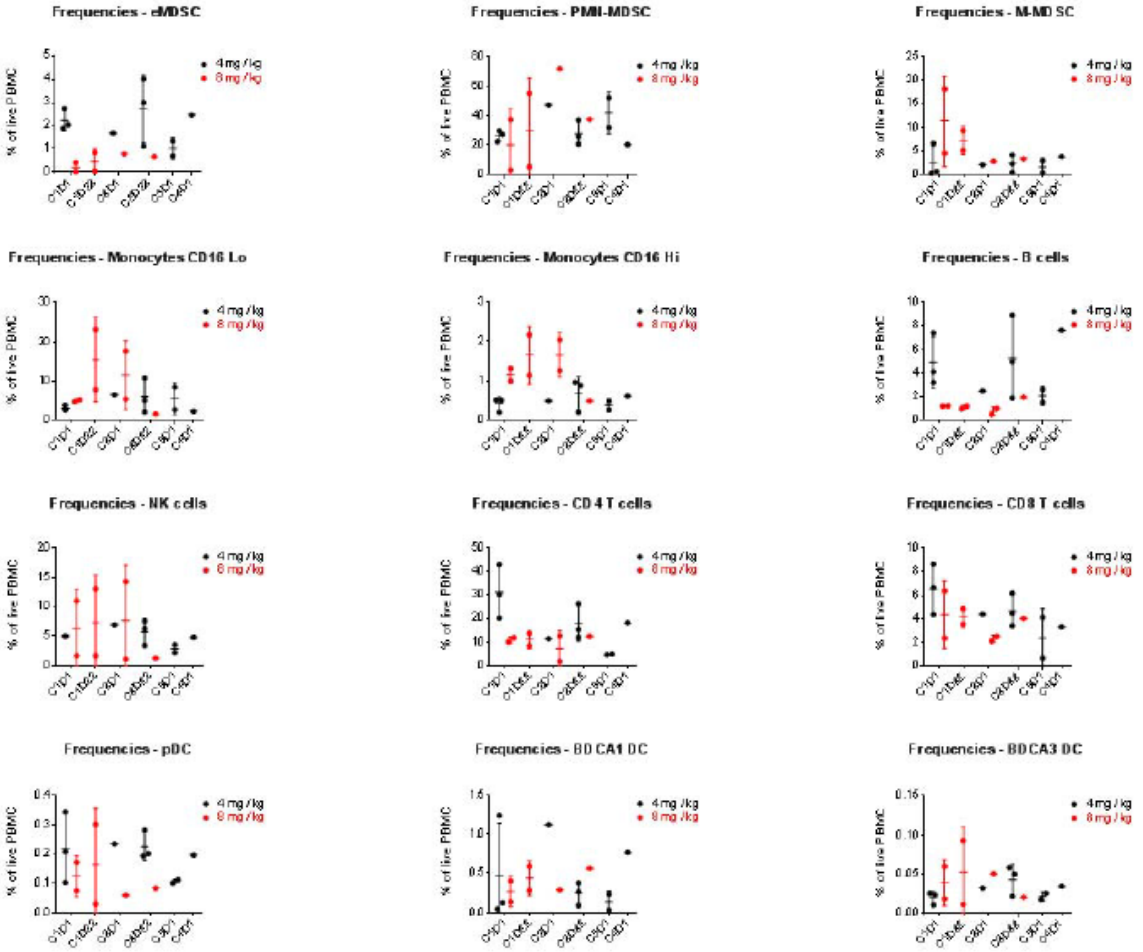

Figure 4. Frequency of myeloid and lymphoid population in peripheral blood of patients treated with different doses of DS-8372a antibody. Results of individual patients are shown.

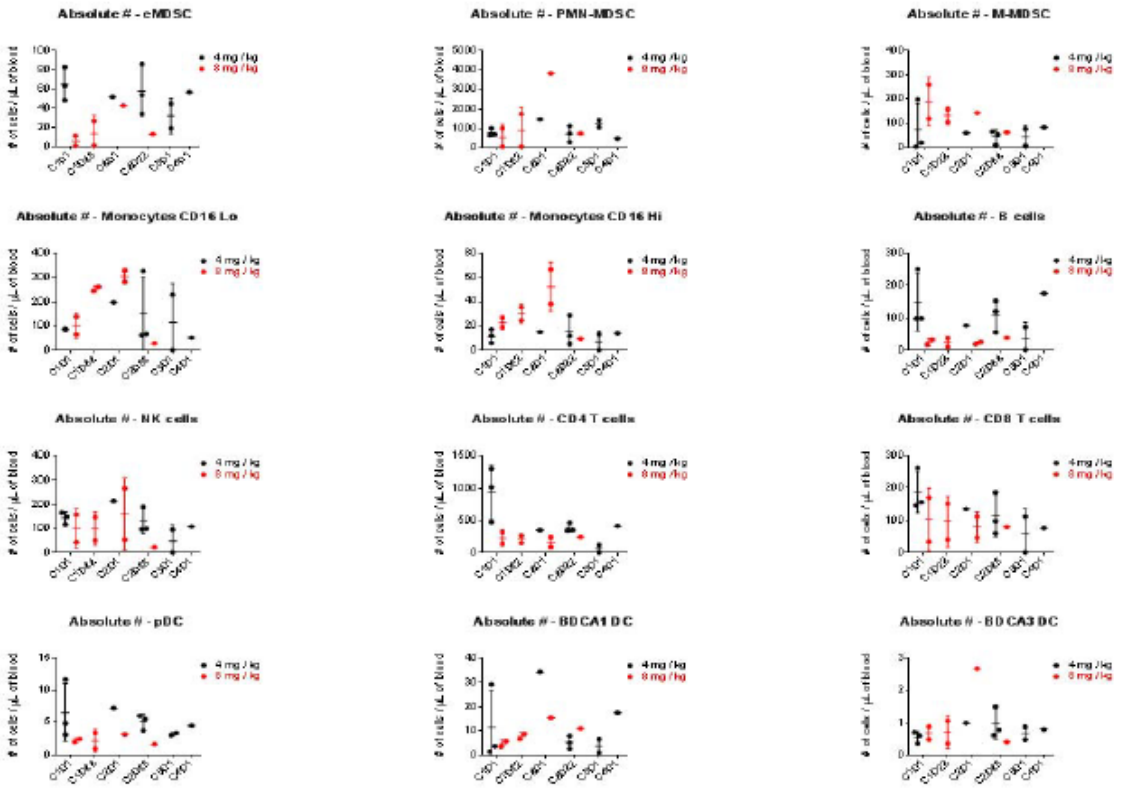

Figure 5. Absolute number of myeloid and lymphoid population in peripheral blood of patients treated with different doses of DS-8372a antibody. Results of individual patients are shown.

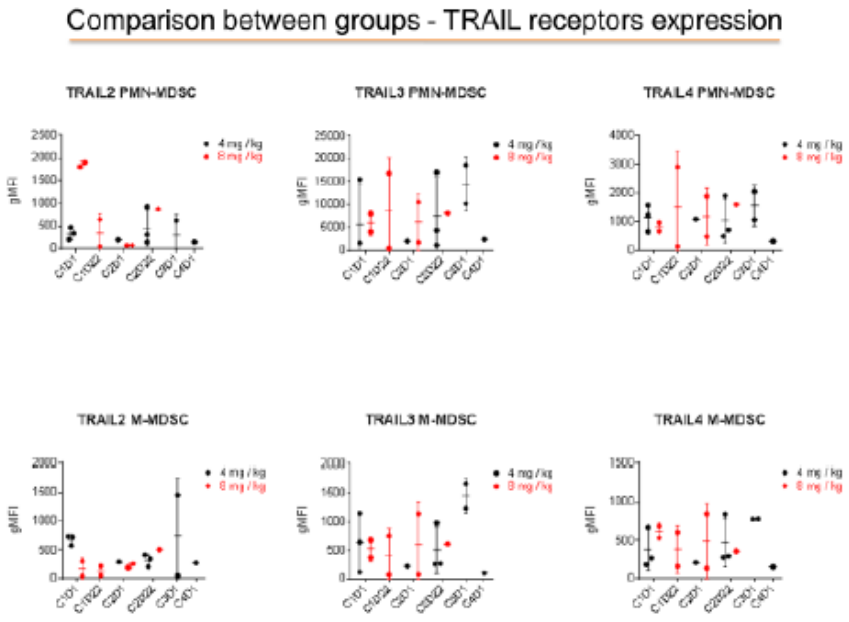

Figure 6. Expression of TRAIL receptors on PMN-MDSC and M-MDSC in patients treated with different doses of DS-8372a antibody. Results of individual patients are shown

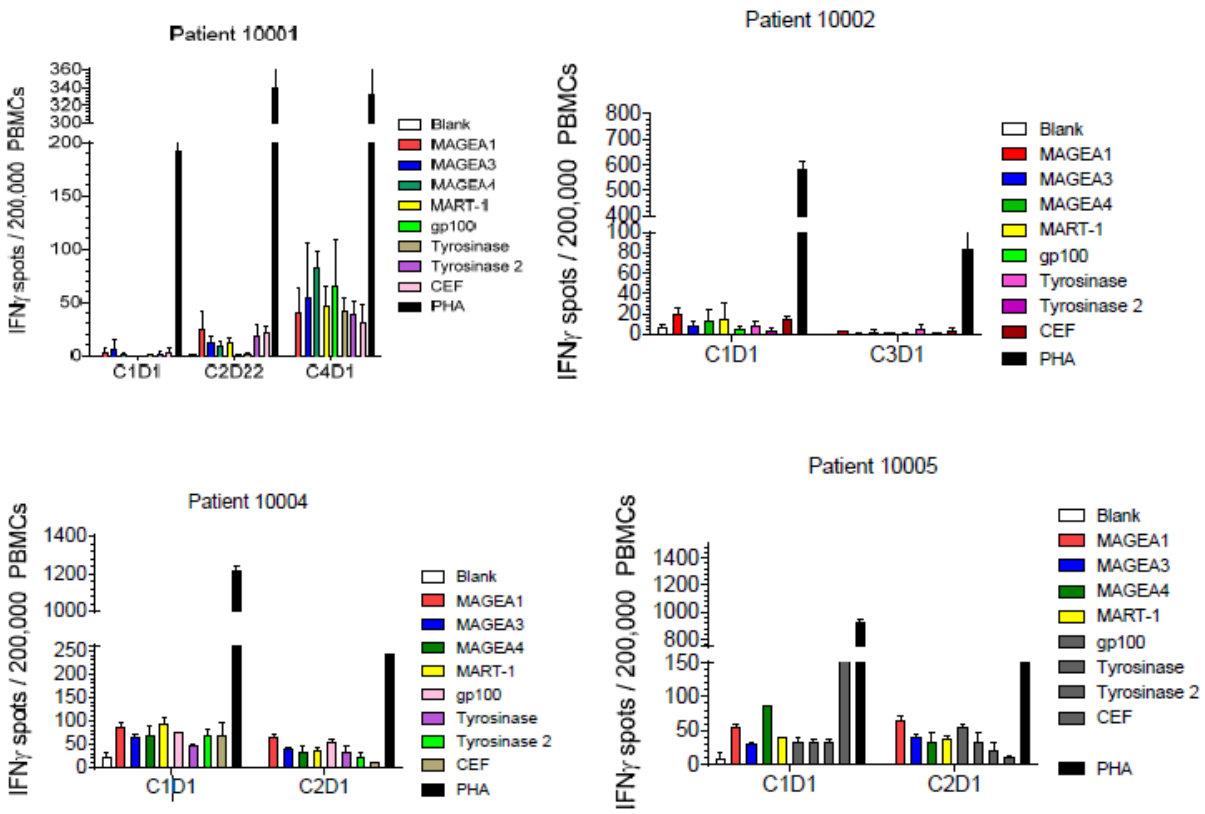

Figure 7. Immune response to melanoma-derived peptides in patients.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

As part of the NYU Science Training Enhancement Program, an 8-week planning course is offered in the Fall and Spring Semesters. This course is required for all 3rd year graduate students and first year postdoctoral fellows. The IDP course exposes trainees to a wide range of career options, assists in completing assessment of individual skills, interests, and values; and guides them through creating an IDP that includes both research and career goals. A written plan is created, and a certificate of completion is printed and returned to the course director, Keith Micoli, Ph.D., who is the Postdoctoral Program Director. The certificate of completion indicates that the trainee has assessed individual skills, values, and interests, explored career options, identified long term career goals, set specific goals for scientific and career advancement, and discussed these goals with their mentors. This is available to the post-doctoral fellow that is part of this proposal at NYU.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Continue to accrue patients, and finish the third cohort, then move onto accrual of the fourth cohort. We will continue to pursue extensive phenotyping analyses of the PBMC from the first 6-9 patients and will send sera from those patients for mass spectrometry analyses to assess associations between proteomic signatures and outcome for the patients. During next funding period we will also continue accumulate data on the effect of the treatment on myeloid population and on melanoma-specific immune response. We expect to complete dose escalating phase of the trial and start expansion cohort. We expect to have enough data to evaluate correlation between the expression of TRAIL-R, ER stress response, and myeloid cell population in patients treated with antibody. We will start assessing functional activity of MDSC in patients.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

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
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No significant changes in project or its direction, but we did amend the clinical protocol (approval attached) to allow a more rapid dose escalation and this did not impact the correlative marker studies that are the subject of this current proposal.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

N/A

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

N/A

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the

reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product,

scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

(1) PD/PIs

Name: **Jeffrey Weber**
Project Role: Principal Investigator - NYU
No change

Name: **Dmitry Gabrilovich**
Project Role: Co-Investigator - Wistar
No change

Name: **Andrew Hu**
Project Role: Co-Investigator - Wistar
No change

(2) Individuals who has worked on the project during the reporting period

Name: Andressa Sodré-Laino
Project Role: Postdoctoral Fellow - NYU
Contribution: No change

Name: Huilin Li
Project Role: Biostatistician - NYU
Contribution: No change

Name: Chih-Hang Tang,
Project Role: Postdoctoral Fellow Hu lab - Wistar
Contribution: No change

Name: **Andong Shao**
Project Role: Postdoctoral Fellow Hu lab - Wistar
Contribution: No change

Name: **Yu-Ji Chen**
Project Role: Technician – Hu Lab - Wistar
Contribution: No change

Name: **George Dominguez**
Project Role: Post-doctoral fellow Gabrilovich lab - Wistar
Contribution: No change

Name: **Jerome Mastio**
Project Role: Post-doctoral fellow Gabrilovich lab - Wistar
Contribution: No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

ACTIVE

Nothing to report

Title: Investigating MGCD0103 as an adjuvant to melanoma immunotherapy
Funding Agency: Mirati Therapeutics
Project Goals: The goal of this study is to investigate the ability of the HDAC inhibitor MGCD0103 to alter the immune response of immune cells and tumor.
Performance Period: : 01/20/2016-12/20/2017
Role: PI
Effort: 0.06 cal
CHANGE: Performance period has been extended. The initial project end date was 1/31/17.

Title: Use of Selective HDACi to Improve Antibody Blockade Immunotherapy
Funding Agency: Melanoma Research Foundation
Project Goals: The central hypothesis to be tested in this application is that manipulation of HDAC6 and/or its molecular partners influence the expression and function of immune checkpoint molecules and other immune-related pathways in melanoma.
Performance Period: 10/01/2016-09/30/2018
Role: NYU Sub-PI (Prime: GW).
Effort: 0.60 cal
CHANGE: Previously "Pending" is now active.

COMPLETED

Title: Investigating the ability of ACY241 to augment cancer immunotherapy
Funding Agency: Acetylon Pharmaceuticals
Project Goals: The goal of this study is to investigate the ability of the HDAC inhibitor ACY241 to augment T-cell function and checkpoint blockade immunotherapies.
CHANGE: At the time of award, this was awarded for a 1-yr project (1/20/16-1/20/17). This project has since ended

foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

New York University
New York, NY
Conducted clinical trial and provided samples of blood for the analysis. Performed correlative analyses

Wistar Institute
Philadelphia, PA
Performed correlative analyses

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.

Overview of study design and status report

NYU Study# S15-00906
Title: A Phase I Study of TRAIL-DR5 antibody DS-8273a administered in Combination with Nivolumab in Subjects with Unresectable Stage III or Stage IV Melanoma
PI: Jeffrey S. Weber MD, Ph.D

Prep. Date: 2017 Aug 03

- Overview of study design
  - Study Design: This is a Phase 1, open label, dose escalation cohort study. The study will consist of a dose escalation assessment of the safety and tolerability of DS-8273a administered concurrently in combination with nivolumab to subjects with advanced melanoma. Treatment will be divided into induction and maintenance phases. It is anticipated that this clinical study will enable selection of the RP2D and dose schedule of this combination for further clinical testing. The trial will include an assessment of the PD activity of DS-8273a administered in combination with nivolumab. A study schematic is presented: Each treatment cycle will be 42 days (6 weeks) during the induction phase, and 84 days (12 weeks) during the maintenance phase. DS-8273a will be administered in escalating doses to successive cohorts of patients. Patients in each cohort will receive DS-8273a intravenously (IV) - on days 1 and 22 of each 42-day induction treatment cycle. The antibody infusion will be over 90 minutes on day 1, and if no infusion reactions are observed, subsequent infusions will be over 60 minutes. Nivolumab will be administered intravenously (IV) at 5 mg/kg over 60 minutes on Days 1 and 22 of each 42-day induction treatment cycle. The induction phase will last for 2 treatment cycles. During the maintenance phase, DS-8273A and nivolumab both will be administered on days 1, 22, 43 and 64 of each 84-day treatment cycle for up to 2 years or until progression of disease, discontinuation due to toxicity, withdrawal of consent or any other reason as specified in Section 3.4 of the protocol.
  - Dose escalation: This is a modification of the traditional 3+3 design. Subjects will be enrolled in successive cohorts of 3. An initial cohort of 3 subjects will be enrolled at the given dose level of DS-8273A, and additional subjects will be added to the same dose level (eg, increase the total number of enrolled subjects from 3 to 6, 9 or 12) based on a modified version of the modified toxicity probability interval (TPI) design. The modified TPI (mTPI) design uses Bayes rule in a decision theoretic framework to determine the MTD based on a pre-specified target toxicity (eg, dose limiting toxicity; DLT) probability pT. A fixed total number of subjects will be used during the dose escalation portion for each cohort. As the dose escalation proceeds under this design, the number of subjects enrolled at a given dose level depends on the number of the toxicities observed at that dose such that multiples of 3 subjects each may be enrolled to enable a dose escalation decision. The MTD is selected as the dose with an observed toxicity rate closest to the pre-specified target toxicity probability PT. In this study, a tight 18% target toxicity (PT) is used. Dose escalation will evaluate DS-8273a at doses ranging from 4 mg/kg to 24 mg/kg mg every 3 weeks administered in combination with nivolumab at doses of 5 mg/kg. The first cohort will receive DS-8273a IV at a dose of 4 mg/kg; nivolumab will be administered IV at a dose of 5 mg/kg every 3 weeks. Enrollment will proceed with administration of escalating doses of DS-8273a in combination with nivolumab as described in Table 1 below:

Table 1 Dose Escalation regimen for DS-8273a and Nivolumab

Dose Cohort	DS-8273a Dose	Nivolumab Dose
1	4 mg/ kg IVQ 3 weeks	5 mg/kg IV Q 3 weeks
2	8 mg/kg IV Q 3 weeks	5 mg/kg IV Q 3 weeks
3	16 mg/kg IV Q 3 weeks	5 mg/kg IV Q 3 weeks
4	24 mg/kg I VQ 3 weeks	5 mg/kg IV Q 3 weeks
-1	2 mg/kg IV Q 3 weeks	5 mg/kg IV Q 3 weeks
-2	4 mg/kg IV Q 3 weeks	3 mg/kg IV Q 3 weeks

Additional dose levels of DS-8273a beyond the 24 mg/kg dose cohort may be added if appropriate based on the safety and tolerability profile of the combination, and after consultation and agreement between Investigators, BMS and Daiichi Sankyo. If cohort 1 shows unacceptable toxicity, then cohort -1, and possibly cohort -2 will be tested as indicated in table 1. There will be no intra-subject dose escalation. Decisions regarding dose escalation between dose levels will be guided by the incidence of drug-related dose limiting toxicities (DLTs) occurring within 42 days (6 weeks; through Day 42 of induction cycle 1) of initiation of study therapy. This observation interval is based upon inclusion of the known median times to onset of common immune-related adverse events attributed to nivolumab (Section 4.2.3.1) and allows for a adequate amount of time for unexpected toxicities with the combined administration of DS-8273a and nivolumab to emerge. Subjects who do not complete the DLT observation period for reasons other than drug-related toxicity will be replaced. There will be a total of 20 patients treated at the RP2D in an expansion cohort to generate additional data on ORR and toxicity.

One of the following five decisions will be made at the end of the DLT evaluation period for each cohort of 3-6 or more subjects if required based on the number of DLTs observed:

S: stay at the same dose and enroll a cohort of 3 more subjects at that dose E: escalate to the next higher dose by enrolling 3 subjects at that dose D: de-escalate to the lower dose, and enroll 3 more subjects at that dose, DU: declare the current dose as unacceptable and unsafe, de-escalate to the lower dose, and enroll 3 more subjects at the lower dose, or C: Study completed and dose is declared to be the MTD

Note that decisions S or E are allowed by the design when determined that the corresponding doses are safe based on the target toxicity. Decisions D or DU are based on evidence of exceeding predetermined toxicity. With a target toxicity rate of 18%, allowing any dose with estimated PT in the (17%, 19%) interval to be selected as the MTD in this study, and with a maximum fixed total of 12 subjects at the dose escalation phase in each cohort, the algorithm for dose escalation decisions based on possible total number of subjects at each dose cohort is described in Table 3.2:

Based on the above design, escalation to the next higher dose level can occur if DLTs are observed in 0 of 3, 1 of 6, 1 of 9, or 2 of 12 subjects, and so on. If DLTs are observed in a greater number of subjects and escalation is not yet permitted, additional subjects in cohorts of 3 will be enrolled at the same dose in order to better estimate the true DLT rate at that dose until either the dose is deemed safe and an escalation is allowed, the dose is deemed unacceptable and should not be used, or de-escalation is recommended, based on decision rules outlined in Table 3.2. At the end of the dose escalation phase, the MTD will be estimated by isotonic

regression or another Bayesian model-based approach (eg logistic regression) using observed toxicities at all studied doses. The combination dose with posterior probability of toxicity closest to the target toxicity, PT, will be declared as the MTD.

The dose escalation plan (see table 1) uses a modified Ji design, with dose escalation decisions made every three patients. The trial will enroll 36 patients for determination of the MTD, but will be successfully concluded earlier if 12 patients are accrued at the dose determined to be the MTD, with at most 3 toxicities.

De-escalation. The dose escalation plan (Table 1) follows a simple order restriction, that is, each dose cohort is as high or higher in both doses compared to all lower cohorts. While cohorts -1 and -2 are both order below cohort 1, their relative unacceptable toxicity rates are unclear. If de-escalation from cohort 1 is needed, it will go to cohort -1. As long as the decisions are to "stay" at the same dose, cohort -1 will be grown to match that of cohort 1, and then they will be alternated. If for the same number of patients treated, the toxicity differential between them reaches 2, the other cohort will be dropped from further study. It is possible that both cohorts could be considered "the MTD". In such an event, other features such as patient response would be used to determine which would be the recommended phase 2 dose (RP2D). The RP2D may be chosen at a dose lower than the MTD if a comparable pharmacodynamics effect on MDSC and anti-tumor immunity is observed at a lower dose of DS-8273a. If the MTD is a unique cohort, that will also be called the RP2D.

This phase I study design was intended to be applied to a fixed sample size of 30. Due to the increased complexity of this combination-dose trial, we have chosen a sample size of 36. An additional expansion cohort totalling 20 patients at the RP2D will be added.

All available clinical and laboratory data, and the nature, time of onset and time to resolution of DLTs observed during dose escalation will be reviewed to determine whether an alternate dose schedule should be examined after consultation between the Investigators, BMS and Daiichi Sankyo if needed. If agreed upon, the alternate schedule will be identified by a protocol amendment and the MTD determined for the revised dose schedule.

In addition to the Investigator's review of study data, the existing Laura and Isaac Perlmutter Cancer Data Safety and Monitoring Committee (DSMC) will monitor the trial as per the NYUCI Institutional Data and Safety Monitoring Plan (version 11-18-11).

- Study objectives
    - Primary Objective: To define safety, tolerability, dose-limiting toxicities (DLTs), maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of DS-8273a administered in combination with nivolumab at 5 mg/kg every 3 weeks in subjects with unresectable Stage III/Stage IV melanoma
    - Secondary Objectives:
        - To describe the preliminary anti-tumor activity of DS-8273a administered in combination with nivolumab to subjects with unresectable Stage III or Stage IV melanoma
        - To monitor Cmax and trough concentrations of DS-8273a administered in combination with nivolumab
        - To evaluate the pharmacodynamics (PD) effect of the combination dose regimen on biomarkers in peripheral blood samples and tumor biopsy specimens

- Exploratory objectives:
  - To explore potential relationships between DS-8273a exposure, biomarker measures, anti-tumor activity and adverse events for the combination dose regimen

- Statistical considerations
  - Study endpoints: Subjects will be required to visit the investigator's office or clinic for physical examinations, vital sign measurements, ECOG performance status evaluation, adverse event assessment, laboratory testing, pharmacokinetic and pharmacodynamic (PD) sample collection, and administration of study drug as per the study schedule.
    - Safety Outcome Measures: All subjects who receive study drug therapy will be evaluated for safety. Safety assessments will be based on medical review of adverse event reports and the results of vital sign measurements, physical examinations, and clinical laboratory tests. Triplicate 12-lead ECGs will be collected prior to dosing on Day 1 of induction cycle 1. Single 12 lead ECGs will be collected at screening, and at the end of treatment. Adverse events will be categorized using the most current version of MedDRA and adverse events and laboratory tests will be graded using NCI Common Terminology Criteria for Adverse Events (CTCAE) v.2.0. Hepatic adverse events will also be recorded relative to ULN. Immune-related adverse experiences among treated subjects will be specifically assessed as well.
    - Efficacy Measures: Tumor response will be determined for all subjects by RECIST 1.1 (see Appendix 3) as well as by immune-related response criteria (irRC; see Appendix 4). RECIST 1.1 will be used for statistical analysis of efficacy in this study. Treatment decisions related to subject management may be based on the irRC criteria, but for the purposes of reporting efficacy RECIST 1.1 criteria will be used. Assessments of tumor status will be made during screening, then at 12 weeks, and subsequently every 12 weeks. Tumor assessments at the end-of-treatment (EOT) or 90-day follow-up visit will be performed only if not assessed within the prior 12 weeks. Individual subject's best overall response (BOR) will be assessed, and progression free survival (PFS) will be calculated by RECIST 1.1 response criteria. Other endpoints are Objective response (OR) defined as BOR outcome of CR or PR for RECIST 1.1, disease control (DC) defined as BOR outcome of CR, PR, or Stable Disease by RECIST 1.1. Duration of response and duration of disease control will also be calculated.

  - Targeted accrual: 36

  - Stopping rules, if applicable: None

  - Duration of monitoring for acute and late toxicities: All SAEs and pregnancies that occur during the course of the study, and for 100 days after drug discontinuation must be reported to the Drug Suppliers (BMS and Daiichi Sankyo) and the FDA within 24 hours

  - Protocol specifications regarding which patients are evaluable for toxicity and response:
    - Male and female subjects ≥ 18 years of age with a histologic or cytologic diagnosis of unresectable Stage III or Stage IV melanoma that are ipilimumab naïve for metastatic disease and who meet the eligibility criteria (see Sections 3.2.1 and 3.2.2) will participate in the study.

- Relevant definitions, such as DLT, SAE and MTD, if applicable
    - **Dose Limiting Toxicity:** For the purpose of guiding dose escalation decision making, hematologic and non-hematologic (hepatic versus non-hepatic) DLT will be defined separately and will be determined based on the incidence, severity, and duration of study drug-related AEs occurring within 42 days (6 weeks; through Day 42 of induction cycle 1) of initiation of study therapy. For the purposes of subject management, DLTs will lead to dose modification regardless of the cycle in which a DLT occurs (see Section 4.2.6 for specific guidelines).
    - **Dose escalation:** This is a modification of the traditional 3+3 design. Subjects will be enrolled in successive cohorts of 3. An initial cohort of 3 subjects will be enrolled at the given dose level of DS-8273A, and additional subjects will be added to the same dose level (eg, increase the total number of enrolled subjects from 3 to 6, 9 or 12) based on a modified version of the modified toxicity probability interval (TPI) design. The modified TPI (mTPI) design uses Bayes rule in a decision theoretic framework to determine the MTD based on a pre-specified target toxicity (eg, dose limiting toxicity; DLT) probability pT. A fixed total number of subjects will be used during the dose escalation portion for each cohort. As the dose escalation proceeds under this design, the number of subjects enrolled at a given dose level depends on the number of the toxicities observed at that dose such that multiples of 3 subjects each may be enrolled to enable a dose escalation decision. The MTD is selected as the dose with an observed toxicity rate closest to the pre-specified target toxicity probability PT. In this study, a tight 18% target toxicity (PT) is used.
    - **De-escalation:** The dose escalation plan (Table 1) follows a simple order restriction, that is, each dose cohort is as high or higher in both doses compared to all lower cohorts. While cohorts -1 and -2 are both order below cohort 1, their relative unacceptable toxicity rates are unclear. If de-escalation from cohort 1 is needed, it will go to cohort -1. As long as the decisions are to "stay" at the same dose, cohort -1 will be grown to match that of cohort 1, and then they will be alternated. If for the same number of patients treated, the toxicity differential between them reaches 2, the other cohort will be dropped from further study. It is possible that both cohorts could be considered "the MTD". In such an event, other features such as patient response would be used to determine which would be the recommended phase 2 dose (RP2D). The RP2D may be chosen at a dose lower than the MTD if a comparable pharmacodynamics effect on MDSC and anti-tumor immunity is observed at a lower dose of DS-8273a. If the MTD is a unique cohort, that will also be called the RP2D

- DSMC review history (provided by CTO):
  May 25, 2017: DSMC Meeting

  Study Progress Report at time of May 25 DSMC Meeting:
  4 patients were accrued. 2 out of 4 patients were taken off treatment due to progressive disease. There were no SAEs. There were no screen failures.

  DSMC Discussion/Meeting Minutes (May Meeting):
  - Dr. Weber gave a brief overview of the study.
    - This is a Phase I study with an expansion cohort of Nivolumab (PD-1antibody) plus TRAIL-DR5 antibody, the protocol is supported by both BMS and Daiichi Sankyo.

- Nivolumab and TRAIL-antibody is given every 3 weeks. Nivolumab is given in fixed dose combination and TRAIL-DR5 antibody is dose expansion depending on which cohort patient are enrolled to.
- No major toxicity has been observed so far. There are 4 patients accrued so far. New patients will be added to the second cohort and then later to third cohort. Then there will be an option to go for expansion cohort.
- Two patients are off study due to progression of disease.

➢ Dr. Cohen presented her review.

- She mentioned there were no AE concerns, the treatment was well tolerated.
- There were no major violations or deviations occurred. Dr. Cohen noted that all 4 patients accrued did not get triplicate EKG's every time and she wanted to know whether this is even necessary or not. Dr. Weber stated that this is a not major violation but should have been done in a timely manner and someone should supervise to make sure the nurses are doing this in a timely manner.
- Dr. Cohen mentioned that timing of Nivolumab varies for administration and Dr. Weber stated that this should have been done in a timely manner as it is explicitly stated in the protocol.
- There was no SAE's on the trial.

➢ DSMC Recommendation: The committee had no concern and stated that the study will be reviewed again at the next reviewing cycle in Aug 2017.

- History of protocol amendments (only major changes, do not include addition/removal of personal) with version dates and brief description of major changes

  - Initial Protocol Version dated 2016 Apr 08
  - (Amendment 1) Protocol Version dated 2017 Feb 02
    - The major change is to shorten the DLT period to 6 weeks from 12 weeks, based on our initial experience of treating 3 patients with virtually no side effects of any kind, and the excellent toxicity track record of the TRAIL antibody DS-8273a. We have built 2 X 6-week induction cycles into the protocol treatment, which remain as before, and will have a natural "stopping point" to assess toxicity after the first 6 week cycle up to the first disease evaluation at week 12.
    - Added clarifications for sample collection, corrected a number of typos, and clarified that patients that have had adjuvant ipilimumab and then relapsed would not be excluded if treated 12 weeks or more after the last dose of that drug.
  - As of 08/03/2017 there have been no major protocol amendments. The only significant changes made have been amendments to the consent based off of updates from the Nivolumab IB (v16 dtd 2017 Jun 23)

- Study progress report:
  - Since the last DSMC review on May 25, 2017, three new patients have signed the consent and 1 out of 3 patients was a screen failure.
  - Total 6 patients accrued on the trial so far, 3 out of 6 patients are off treatment due to progressive disease, 3 patients are active and on treatment
  - No SAEs are reported on the trial as of now.

NYU School of Medicine
NYU LANGONE MEDICAL CENTER

Office of Science and Research
Institutional Review Board
1 Park Ave.| 6th Floor, | New York, NY 10016

Approval of Submission September 20, 2017

Dear Dr. Jeffrey Weber:

On 9/19/2017 4:00 PM EDT, the IRB reviewed the submission below: All conditions for approval were met on 9/19/2017.

principal investigator	Jeffrey Weber
email	Jeffrey.Weber2@nyumc.org
study number	i16-01975
study title	Correlative Studies on A Phase 1 Study of TRAIL-DR5 antibody DS-8273a Administered in Combination with Nivolumab in Subjects with Unresectable Stage III or Stage IV Melanoma s15-00906
performance period	9/19/2017 - to 12/30/2017 inclusive. Before 12/30/2017 or within 30 days of study closure, whichever is earlier, you are to submit a continuing review with required explanations. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR. If continuing review approval is not granted before the expiration date of 12/30/2017, approval of this study expires on that date.
location(s)	Clinical Cancer Center (NYUMC Locations), Tisch Hospital (NYUMC Locations)
sponsor(s)	Name: Department of Defense (DOD) - Army;
review type	Modification
board name	
materials approved for use	The following personnel change has been noted: AddingJennifer Tiao as a Regulatory Specialist Removing Merle Henry
#of subjects approved to consent	36

The current IRB Status of your study is: Approved . This study was reviewed by the NYU School of Medicine's Institutional Review Board (IRB). During the review of your study, the IRB specifically considered:

1. the risks and anticipated benefits (if any) to your subjects
2. the selection of subjects
3. the procedures for securing and documenting informed consent
4. the safety of your subjects
5. the privacy of your subjects and confidentiality of the data

Your study cannot commence until all ancillary review decisions are complete. In order to determine the state of all ancillary reviews please go the My Studies page of this study in Research Navigator. Ancillary review statuses will be found on the right side of the header section.

Please note; if your study includes a clinical trial agreement or budget you will need to ensure approval has been issued from My Agreements/CRMS and The Office of Clinical Trials before you proceed with any aspects of this study including the enrollment of human subjects.

Review Notes

For NIH Grant funded research: the IRB has found the IRB approved protocol referenced above to be consistent with the NIH grant application.

Helen Panageas September 20, 2017

RE: Study#i16-01975

Helen Panageas, Director, Institutional Review Board OHRP #FWA00004952

Notes

- You must submit all changes to this study (e.g., protocol, recruitment materials, consent forms, etc.) via eSubmission to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB within 24 hours.
- You must report all adverse and/or unanticipated event(s) that occur during the course of this study to IRB via eSubmission in accordance with IRB Policy.
- Use only IRB-approved copies of your consent form(s), questionnaire(s), letter(s), advertisement(s), etc. in your study. Do not use expired consent forms.
- You must inform all research staff listed on this study of changes or adverse events that occur.
- IRB's approval is valid until the end date of the performance period indicated above. A reminder for renewal should be e-mailed to you from the IRB 90, 60 and 30 days before this study's approval is scheduled to expire. However, you are responsible for submitting all renewal materials at least eight weeks before expiration regardless of whether or not you receive a reminder notice.
- All IRB policy documents can be found on our website: http://irb.med.nyu.edu/library
- Prior to initiating an IRB-approved study, you must receive written approval from an authorized representative for each site where your study will take place. Key contacts are:
    - **Bellevue Hospital**: when Bellevue Hospital is listed as a site where your study can take place, please note that you may have to complete additional work in BHC's Reason system. Bellevue will be contacting you with any additional needed information. For questions on Bellevue Hospital research, please contact *BellevueResearch@bellevue.nychhc.org*
    - CTSI - Clinical and Translational Science Institute, NYU School of Medicine [formerly General Clinical Research Center (GCRC)], ctsi@nyumc.org.
    - NYU Langone Medical Center (Tisch Hospital/Rusk Institute/Co-op Care/HJD/Perlmutter Cancer Center) site approval is handled for you automatically (as needed) by the Office of Clinical Trials
- The IRB may terminate studies that are not in compliance with NYU Langone Medical Center/School of Medicine Policies & Procedures and the requirements of the Institution's Federal Wide Assurance with the Federal Government. Direct IRB questions, correspondence and forms (e.g., continuing reviews, amendments, adverse events, etc.) to 212-263-4110 or IRB-INFO@nyumc.org.
- Prior to initiating an IRB-approved study, you must receive written approval from an authorized representative of the Office of Clinical Trials. You may contact the Office of Clinical Trials at 212.263.4210 or clinicaltrials@nyumc.org.

NYU SoM IRB operates in accordance with Good Clinical Practices (GCP) and applicable laws and regulations. The NYU SoM IRB Federal Wide Assurance number is 00004952.