OMB Number:
Expiration Date:

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

2. DATE SUBMITTED | **Applicant Identifier**

4. a. Federal Identifier

b. Agency Routing Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** 004514360

* Legal Name: University of Pittsburgh

Department: Office of Research Division:

* Street1: 123 University Place

Street2: University Club, Lower Lobby

* City: Pittsburgh County / Parish: Allegheny

* State: PA: Pennsylvania Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 15213-2303

Person to be contacted on matters involving this application

Prefix: Ms. * First Name: Debra Middle Name:

* Last Name: Evansky Suffix:

* Phone Number: 412-624-7400 Fax Number: 412-624-7409

Email: offres@offres.pitt.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 1250965591A6

7. * TYPE OF APPLICANT: X: Other (specify)

Other (Specify): Private Non-Profit State Related Educ. Institution

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☒ New ☐ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Stimulatory and Suppressive NK Cell, DC, and MDSC Interactions in Human Cancer

12. PROPOSED PROJECT:
* Start Date: 12/01/2011 * Ending Date: 11/30/2015

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT**
PA-014

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: * First Name: Jeffrey Middle Name: Ling-Yi

* Last Name: Wong Suffix:

Position/Title: MD/PhD Candidate

* Organization Name: University of Pittsburgh

Department: Surgery Division: Surgical Oncology

* Street1: 5117 Centre Ave

Street2:

* City: Pittsburgh County / Parish: Allegheny

* State: PA: Pennsylvania Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 15213-1863

* Phone Number: 412-623-3251 Fax Number: 412-623-7709

* Email: wong.jeffrey@medstudent.pitt.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	295,842.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	295,842.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: Mr. * First Name: Allen Middle Name: A.

* Last Name: DiPalma Suffix:

* Position/Title: Director, Office of Research

* Organization: University of Pittsburgh

Department: Office of Research Division:

* Street1: 123 University Place

Street2: University Club, Lower Lobby

* City: Pittsburgh County / Parish: Allegheny

* State: PA: Pennsylvania Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 15213-2303

* Phone Number: 412-624-7400 Fax Number: 412-624-7409

* Email: offres@offres.pitt.edu

*** Signature of Authorized Representative**: Allen DiPalma

*** Date Signed**: 04/04/2011

20. Pre-application Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific Table Of Contents

OMB Number:
Expiration Date:

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Pittsburgh

DUNS Number: 0045143600000

* Street1: 123 University Place

Street2: University Club, Lower Lobby

* City: Pittsburgh County: Allegheny

* State: PA: Pennsylvania

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 15213-2303 * Project/ Performance Site Congressional District: PA-014

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City: County:

* State:

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: * Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☒ Yes ☐ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☒ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☒ No

IRB Approval Date: 03/18/2011

Human Subject Assurance Number: 00006790

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract Project_Summary_JLW_F30.pdf Add Attachment Delete Attachment View Attachment

8. * Project Narrative Project_Narrative_JLW_F30.pdf Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited Bibliography_and_References_Cited_JLW Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources Facilities_and_Other_Resources_JLW_F3 Add Attachment Delete Attachment View Attachment

11. Equipment Equipment_JLW_F30.pdf Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments ☒

Project Summary

Natural killer (NK) cells have been recently implicated as critical modulators of adaptive immunity. In particular, NK cell interactions with dendritic cells (DCs) are central to shaping effective anti-cancer immune responses, holding great implications for DC- and other cell-based cancer immunotherapies. We have previously demonstrated that, in contrast to IL-2-activated 'killer' NK cells capable of eliminating immune-stimulatory DCs, IL-18-activated 'helper' NK cells can potentiate anti-tumor immune responses through DC activation and the enhancement of DC-induced type-1 immunity. However, our new preliminary data indicate that such IL-18-activated NK cells may also have undesirable immune-suppressive functions through the hyper-activation of myeloid-derived suppressor cells (MDSCs), reinforced by autocrine COX2-PGE_2 feedback in MDSCs. This further suggests the possibility of COX2-PGE_2 axis inhibition in reversing the NK-mediated up-regulation of MDSC functions, while preserving or enhancing NK-mediated DC activation.

In this proposal, using IL-2- and IL-18-activated NK cells as a model, I seek to identify the mechanisms by which 'killer' and 'helper' NK cells differentially acquire and perform desirable immune-stimulatory (tumor-killing, MDSC-killing, and DC-activating) and undesirable immune-suppressive (DC-killing and MDSC-activating) functions. This mechanistic knowledge will be subsequently used to examine potential pharmacologic or biologic methods of modifying NK cell interactions with DCs and MDSCs to maximize desirable anti-cancer immune responses. Overall, this project will provide new functional and mechanistic insights into the acquisition and performance of NK cell immune-stimulatory and immune-suppressive interactions with DCs and MDSCs, and will identify targets for the therapeutic separation of these desirable and undesirable NK cell activities for the improvement of cancer immunotherapy.

Project Narrative

Cancer remains the leading cause of death under age 85 in the US, highlighting the need for innovative approaches to cancer treatment, including strategies to enhance the effectiveness of immune responses against cancer. Using highly-relevant human tumor models and clinical cancer materials, this proposal seeks to define the key interactions between three cell types critical to the immune response and immune evasion of cancer: natural killer (NK) cells, dendritic cells, and myeloid-derived suppressor cells. The results of this study will directly contribute to the improvement of cell-based cancer therapies by informing strategies for the selective enhancement of immune-stimulatory anti-cancer NK cell activities.

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phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors NKp30 and NKp46. Eur J Immunol. 2001;31:1656-1665.
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Facilities & Other Resources

Laboratory

The sponsor occupies approximately 1,200 sq. ft. of laboratory space on the first floor of the Research Pavilion of the Hillman Cancer Center (HCC) Building. This laboratory space is fully-equipped with all research resources necessary for the success of the applicant's proposal, and includes further full access to the extensive on-premise core facilities of the University of Pittsburgh Cancer Institute (UPCI), including advanced biologic imaging facilities (see section on "Equipment" for a detailed description of all available core facilities). The laboratory is situated within the larger HCC Research and Clinical Complex of the UPCI, a unique environment housing in one location more than 20 fully-functional basic and translational cancer research laboratories as well as diverse clinical and education/training facilities. This environment will strongly contribute to the research and professional development of the applicant by facilitating interaction with a large number of leading cancer-focused scientists, an extensive base of clinical cancer faculty, and the rich training infrastructure of the UPCI.

Clinical

As described above, the sponsor's laboratory is situated within the unique HCC research/clinical complex, which houses the comprehensive clinical activities of the UPCI within a five-story, 150,000 sq. ft. facility. Clinical services include cancer treatment, diagnosis, prevention and early detection, nutrition, education, genetic counseling, and behavioral medicine support. The close proximity of this clinical infrastructure to the HCC research complex facilitates routine translational research interactions between research and clinical faculty and physician-scientist integration of their research and clinical activities. The applicant's research, clinical, and professional development will benefit strongly from this close association, including access to diverse physician-scientist faculty (also facilitating the success of longitudinal clinical clerkships during graduate study) and access to clinical cancer materials for the completion of the proposed studies. In a similar manner, the applicant will also benefit from the sponsor's close association with the clinical and research resources of the Magee-Womens Hospital (MWH). MWH is one of the largest free-standing obstetrics and gynecology hospitals in the country with a robust gynecologic oncology program (treating over 100 newly-diagnosed ovarian cancers per year), offering unique expertise and access to clinical cancer material for the success of the applicant's proposal.

Office and Computer

The applicant has dedicated access to an office workspace within the sponsor's laboratory, situated directly adjacent to the sponsor's office. Available computer resources offer full access to hardware and software necessary for data entry and analysis pertinent to this proposal, including sole use of a Hewlett-Packard Core 2 Quad computer linked to the university main frame computer system, access to a lab-shared Dell Core i7 computer with additional data analysis software, and access to hardware and software as needed for specialized applications in core-facility computers (such as intensive image processing and analysis).

Additional Intellectual Resources

The applicant will benefit from statistical support through the Biostatistics Core of the UPCI, which provides statistical and computer-related expertise in design, execution, analysis, and reporting of cancer-related research studies. The applicant will also benefit from intellectual expertise in clinical and translational research design, analysis, and reporting through the University of Pittsburgh's Institute for Clinical Research Education and the Clinical and Translational Science Institute. These resources will allow for the comprehensive development of research skills necessary for a future career in translational cancer research.

Equipment

The applicant will have full access to all laboratory equipment necessary for the completion of the proposed studies. The sponsor's laboratory is fully equipped for cellular immunology research and available to the applicant: 4 laminar flow hoods, 4 CO_2 incubators, 3 adjustable speed/temperature cell centrifuges, 3 inverted microscopes, ELISA washer, PCR cycler (Taqman), water bath incubators, a histologic microscope, 2 Eppendorf centrifuges, 3 scales, 2 -80°C freezers, 2 -20°C freezers, 2 -4°C refrigerators, other minor laboratory equipment, and the necessary software and hardware for data analysis, including for flow cytometry, quantitative PCR, and microscopic imaging. Shared facilities in immediate proximity provide additional unrestricted access to equipment for ELISA and ELISPOT analysis, liquid nitrogen long-term cryopreservation tanks, gamma and beta counters and other equipment necessary for radioactive experimentation, HPLC equipment, a cryostat for preparation of histological slides, cell harvesters, and additional centrifuges (high speed- and ultra-centrifuges). Space and fixtures include an additional molecular biology room with fume hood, molecular histopathology room, dark room, microscopy/imaging room, and walk-in cold room. As part of the University of Pittsburgh Cancer Institute, the applicant will have further full access to its extensive core facilities (most pertinent to this proposal are listed below):

- Flow and Imaging Cytometry Core (adjacent to the lab): contains equipment for 4- and 12-color flow cytometry analysis, cell-sorting, and a Cellomics Arrayscan HCS reader (Thermo Scientific) for quantifying the sub-cellular fluorescence of targets of interest
- Biologic Imaging Satellite Facility (adjacent to the lab; directed by Dr. Per Basse, MD, PhD, a member of the applicant's thesis committee): contains multiple light microscopes, a temperature- and CO_2-controlled time-lapse imaging microscope, Leica, Olympus, and Zeiss confocal and two-photon microscopes, and microtomes
- Luminex Facility: provides a Luminex Bio-Plex workstation (Luminex 100) for the analysis of cytokine and chemokine profiles
- Vector Core: provides viral vectors (adenovirus, retrovirus) for gene therapy applications
- Proteomics and Mass Spec Core: provides cytogenetics, DNA sequencing, DNA and RNA isolation, Serial Analysis of Gene Expression (SAGE) library production, SNP genotyping, STRP genotyping, microarray analysis (Affymetrix, CodeLink, and custom arrays), quantitative RT-PCR, Difference Gel Electrophoresis, Ciphergen Proteinchip Profiling, protein identification and sequencing, and state-of-the-art molecular analysis of biomolecules
- Peptide Synthesis Facility: comprehensive services for peptide synthesis, purification, and characterization for research programs at the University of Pittsburgh and UPMC
- Tissue and Research Pathology Services: provides centralized tissue and biological specimen procurement services, research histology services, annotated clinical data, and tissue microarray services
- IMCPL facility: provides therapeutic cell product generation (CPL) and serial monitoring of immunologic functions in patients with cancer who are treated with biologic therapies (IML)
- Biostatistics Core: provides statistical and computer-related expertise in design, execution, analysis, and reporting of cancer-related research studies

The applicant will have further access to the Center for Biologic Imaging (directed by Dr. Simon Watkins, PhD, a member of the applicant's thesis committee), which encompasses 17 confocal microscopes, 2 TEM microscopes, 1 SEM microscope, 17 additional assorted microscopes for various applications, extensive imaging processing and analysis hardware and software, miscellaneous additional equipment to support sample imaging (including live cell chambers and syringe pumps for media perfusion), and biologic imaging expertise of dedicated staff. Additional institutional intellectual resources will also be available to him, including clinical research expertise through the Institute for Clinical Research Education and the Clinical and Translational Science Institute.

Additional Educational Information

1. Standardized Exam Scores

MCAT Score:
USMLE Step 1 Score:

2. Applicant's Course Grades

See Fellowship Applicant Biographical Sketch, "Scholastic Performance" section.

3. Description of Combined Degree Program

Medical Scientist Training Program (MSTP) of the University of Pittsburgh and Carnegie Mellon University

Led by Dr. Clayton Wiley, MD, PhD and co-directed by Dr. Richard Steinman, MD, PhD and Dr. George Stetten, MD, PhD, the MSTP is a well-structured MD-PhD dual-degree training program specifically tailored for the development of future physician-scientists participating in biomedical research, through the close integration of graduate research and graduate medical study. The MSTP jointly encompasses the extensive faculty, institutional resources, and training programs of both the University of Pittsburgh and Carnegie Mellon University, with considerable support from both the NIH and the University of Pittsburgh School of Medicine.

The program has a unique curriculum integrated throughout both the research and clinical training phases for the longitudinal development of research, clinical, professional, and ethics skills. In the summer prior to matriculation, students participate in the first of three laboratory research rotations intended to begin/continue developing research and professional skills in biomedical research and aid in the identification of potential thesis advisors for graduate training. Additional research rotations are scheduled between the first and second years of medical school and optionally between the second year of medical school and the beginning of full-time graduate study. The summer before matriculation also includes an MSTP-specific course entitled "Molecular Medicine," meeting 1.5 hours per week for 8 weeks, in which faculty from across the School of Medicine and Graduate School of Public Health present their departments, affiliated research, and relevant scientific techniques to introduce students to new scientific opportunities and approaches.

During the first two years of medical school, MSTP students participate in the "Research Basis of Medical Knowledge" courses, which use a scientific journal club format (guided by rotating expert faculty) to develop skills in reading, interpreting, critiquing, and presenting seminal papers in the biomedical sciences, bridging medical school coursework and the research breakthroughs detailed in the basic science blocks. The preclinical medical curriculum includes courses on fundamental principles in anatomy, embryology, biochemistry, genetics, molecular biology, microbiology, immunology, pathology, pharmacology, organ system-based physiology and pathology, physician skills (patient interviewing, physical exam/diagnosis), behavioral medicine, and health policy. Following the second year of medical school, prior to full-time graduate training, MSTP students complete 8 weeks of required clinical clerkships, providing a foundation for translational thought during graduate studies and a basis for the future completion of two Longitudinal Clinical Clerkships during graduate research work (20 week, half-day per week outpatient clinical rotations closely-mentored by a physician mentor, providing clinical continuity, skill development, and refinement of clinical interests during graduate training).

Graduate study in the Department of Immunology works closely with the MSTP to support the training of physician-scientists. Introductory course requirements are satisfied by prior medical coursework, allowing more time for advanced and focused elective coursework and a quicker transition to majority-time research training. The departmental Comprehensive Exam and thesis proposal are typically completed during the second year. Yearly presentation by graduate researchers is structured into the department-wide Immunology Seminar. During graduate study, MSTP students also complete MSTP-specific courses on professional development (focusing on scientific writing, presentation, and networking) and ethics (particularly related to issues uniquely encountered by physician-scientists). Following dissertation defense, students transition back to the remaining third and fourth clinical years of medical school. During this period, elective research rotations help maintain a connection to dissertation work or allow for involvement in new (often clinical) research projects.

Additional aspects of the MSTP include semi-annual meetings with a formal MD-PhD Career Advisor, providing specific guidance on dual-degree training and research and clinical balance. Students also participate in the monthly MSTP Seminar/Workshop series. This series addresses scientific, professional, and ethics issues particularly important to future physician-scientists, and also offers structured interaction with additional physician-scientist mentors. MSTP students also participate annually in the MSTP Retreat, a student-organized, program-filled event that further provides a structured opportunity to present research and engage in scientific and professional development with peers and physician-scientist role models.

List of Referees

1. Michael Lotze, MD (Professor of Surgery, Immunology, and Bioengineering, University of Pittsburgh and University of Pittsburgh Cancer Institute; Vice Chair of Research, Department of Surgery, University of Pittsburgh; Director of Strategic Partnerships, University of Pittsburgh Cancer Institute)
2. Richard Steinman, MD, PhD (Associate Professor of Medicine and Pharmacology, University of Pittsburgh and University of Pittsburgh Cancer Institute; Co-Director, Medical Scientist Training Program, University of Pittsburgh)
3. Robert Ferris, MD, PhD (Professor of Otolaryngology, Immunology, and Radiation Oncology, University of Pittsburgh and University of Pittsburgh Cancer Institute; Chief, Division of Head and Neck Surgery, Department of Otolaryngology, University of Pittsburgh; Co-Leader, Cancer Immunology Program, University of Pittsburgh Cancer Institute)

.

Section II – Sponsor and Co-Sponsor Information

II.A. Research Support Available

Current Research Support		
P01 CA101944 (NIH/NCI) "Integrating NK and DC into Cancer Immunotherapy" Role: PI of Project 2	**(Lotze/Kalinski)**	**07/06/2005 – 06/30/2011** $1,243,053
R01 CA095128 (NIH/NCI) "Regulation of DC Activity by Memory and Effector $CD8^+$ T cells" Role: PI	**(Kalinski)**	**07/01/2010 – 06/30/2011** $537,066
P01 CA132714 (NIH/NCI) "Directing Tumor-Specific T cells to Tumors" Role: Overall Program PI, PI of Project 1 and Core A, Co-I on Project 3	**(Kalinski)**	**04/27/2009 – 03/31/2014** $717,288
R01 CA134633 (NIH/NCI) "Clinical Translation of 19F MRI to Visualize Cancer Immunotherapeutic Cells" Role: PI (MPI)	**(Ahrens/Kalinski)**	**05/10/2009 – 04/30/2012** $1,200,000
P50 CA121973 (NIH/NCI) "SPORE in Skin Cancer" Role: PI of Project 3	**(Kirkwood/Falo)**	**08/26/2008 – 07/31/2013** $2,300,000
P01 CA109688 (NIH/NCI) "Immune Escape of Human Cancers: Cellular Mechanisms and Countermeasures" Role: Co-I	**(Whiteside)**	**09/30/2006 – 07/31/2011** $1,071,960
Pending Research Support		
P01 CA101944 (NIH/NCI) "Integrating NK and DC into Cancer Immunotherapy" Role: Overall Program Co-PI (MPI), PI of Project 2, Co-PI of Core A	**(Lotze/Kalinski)**	**10/1/2011 – 09/30/2016** $1,091,814

II.B. Sponsor's Previous Fellows/Trainees

Past Trainees in Kalinski Lab (selected from 15 previous trainees)			
Name	**Training Period**	**Project**	**Current Position**
Je-Jung Lee, MD, PhD	2006 - 2007	DC1-based vaccines in hematologic malignancies	Associate Professor of Hematology-Oncology, Chonnam National University, Gwangju, Korea; Director, Research Center for Cancer Immunotherapy, Chonnam National University Hwasun Hospital, Jeollanamdo, Korea
Young-Ik Son, MD, PhD	2000 - 2001	1) IL-12 production by $CD8a^+$ and $CD8a^-$ DC in mice; 2) synergistic anti-tumor activities of IL-12 and IL-18	Associate Professor, Department of Otorhinolaryngology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea
Yutaro Nakamura, MD, PhD	2003 - 2005	Helper function of mouse $CD8^+$ T cells	Assistant Professor, Second Dept Internal Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan
Adam Giermasz, MD	2001 - 2005	Polarized dendritic cells as a tool to induce anti-cancer immunity	Fellow, Division of Hematology/Oncology, Department of Medicine, University of California San Francisco
Robbie Mailliard, PhD	2002 - 2006	Helper role of $CD8^+$ T cells and NK cells in the induction of DC-mediated Type-1 immunity.	Research Assistant Professor, Department of Infectious Disease and Microbiology, University of Pittsburgh

II.C. Training Plan, Environment, Research Facilities

II.C.1. Training Plan

Graduate Coursework:

Jeff has and will continue to benefit from the extensive curricular resources of the University of Pittsburgh to develop both an enhanced conceptual knowledge of immunology and cancer biology as well as specific practical knowledge in research design and techniques. By the start of this fellowship period, Jeff will have completed the vast majority of his Immunology Graduate Program coursework. This includes advanced didactic courses on Comprehensive Immunology (examining advanced immunologic concepts in immune cell

development, recognition, signaling, and interactions) and on Immunology in Health and Disease (examining immune paradigms in human health, including cancer immunology). Jeff will have also completed a course on the Experimental Basis of Immunology, an intensive didactic/workshop course analyzing contemporary experimental methods. Furthermore, Jeff will have also completed an elective in Clinical and Translational Research Methods, offered through the Institute for Clinical Research Education, which will facilitate his understanding of trial-oriented research, including study design, ethics, and statistical analysis. These skills will be very relevant to his future role as an independent investigator closely involved with clinical studies.

During the award period, Jeff will complement this completed coursework with a dedicated course on Cancer Biology & Therapeutics, which will explore in detail the current and emerging paradigms and treatment approaches in cancer, including a block dedicated to cancer immunotherapy. This course will provide a sound didactic basis for a future career in developing translational cancer immunotherapies. As relevant, Jeff will also take unique advantage of short, non-credit courses in microscopic imaging techniques offered through the renowned University of Pittsburgh Center for Biologic Imaging, which will facilitate both the completion of his fellowship project as well as establish a foundation in imaging skills highly relevant to investigation in this field.

Research Experience:

Throughout this fellowship, Jeff will learn how to function as an independent investigator while concurrently building a strong base of scientific and technical knowledge for a future career in translational cancer research. Completion of his research proposal will provide a strong vehicle for acquiring essential knowledge of contemporary techniques in immunologic research, including a diverse range of genetic, protein, and cellular assays. Jeff will also gain a strong working knowledge of biologic imaging techniques, supported by the mentorship and expertise of Dr. Simon Watkins, PhD (a member of Jeff's thesis committee and Director of the Center for Biologic Imaging) and members of his group. His research proposal will also provide an avenue for understanding the acquisition and use of human clinical materials, essential to translational cancer research. His development of critical research thought, experimental design, and scientific communication (both in manuscripts and poster and oral presentations) will continue to be developed through multiple avenues described in detail below, including close and frequent interactions with myself and interactions with his thesis committee, members of the broader University of Pittsburgh environment, and the cancer research community.

Intramural Meetings and Seminars:

I meet with Jeff weekly on an individual basis and will continue to do so throughout his training. In these meetings we discuss in detail his ongoing and future research, including technical approaches, analysis of primary data, and conceptual implications. These meetings also regularly focus on professional development. For instance, we often discuss meeting abstract and manuscript drafts to help focus his work, assess future experiments, and gain perspective on how his work might be evaluated by outside reviewers. We also discuss opportunities to present his work to diverse audiences, whether at institutional meetings or larger conferences. Jeff will also benefit from our weekly lab meetings, which broaden his mentorship and refinement of scientific ideas, particularly during presentation of his work to the group approximately every two months.

Jeff completed his PhD Comprehensive Examination in December 2010 and has since formed and met with his dissertation committee, which consists of:

- Pawel Kalinski, MD, PhD (Committee chair and dissertation advisor; Professor of Surgery, Immunology, and Infectious Disease & Microbiology; Director of Research, Division of Surgical Oncology)
- Robert Edwards, MD (Professor and Executive Vice Chair of Obstetrics, Gynecology, and Reproductive Sciences; Director, Ovarian Cancer Center of Excellence)
- Michael Lotze, MD (Professor of Surgery, Immunology, and Bioengineering; Vice Chair of Research, Department of Surgery; Director of Strategic Partnerships, University of Pittsburgh Cancer Institute)
- Walter Storkus, PhD (Professor of Dermatology and Immunology)
- Simon Watkins, PhD (Professor and Vice Chair of Cell Biology & Physiology; Director, Center for Biologic Imaging)
- Per Basse, MD, PhD (Associate Professor of Immunology)

Jeff's committee members are experts in their respective fields and collectively offer an incredible resource for basic and translational scientific knowledge (including NK cell and myeloid cell biology), clinical and clinical trial experience, and technical expertise (including advanced biologic imaging) that will contribute strongly to Jeff's dissertation work and development as a translational scientist. Meetings with this entire group will occur formally every 6 months, in which his dissertation progress will be reviewed in detail. More frequent meetings with individual members will be available as needed for technical, scientific, and/or career advice.

Jeff will also benefit from semi-annual formal and more frequent informal meetings with his MSTP Career

Advisor, Dr. Gary Silverman, MD, PhD (Professor of Pediatrics and Cell Biology & Physiology; Chief, UPMC Newborn Medicine; Director, Neonatal-Perinatal Training Program). Dr. Silverman offers particular expertise in mentoring students on research/clinical balance, MD/PhD training, and physician-scientist career milestones.

In addition, his participation and presentation at numerous intramural seminars will provide critical and timely exposure to contemporary topics in the field and enhanced interactions with peers and experts, supporting the development of his collaborative skills. These seminars include the Immunology Department seminar, the University of Pittsburgh Cancer Institute (UPCI) Tumor Immunology Series, the UPCI Cancer Biology & Immunology journal club, and the Magee-Womens Research Institute monthly seminar. Additionally, Jeff will participate in the UPCI's monthly Cancer Immunology, Immunotherapy, and Immunoprevention Program meeting, which will provide first-hand experience with investigative integration across multiple groups in both research and clinical spheres. Jeff will also present yearly at the annual retreats of the UPCI, which will continue to foster his scientific communication skills as well as scientific interactions at an institutional level.

Extramural Interactions:

Jeff's training will heavily incorporate interactions outside the University of Pittsburgh, which will allow the diversification of his conceptual exposures, refine his oral and written communication skills, and facilitate connections with individuals and institutions highly relevant to his future goal of becoming an independent researcher in the cancer immunotherapy field. Specifically, his presentations at the meetings of the American Association of Cancer Research and the Society for Immunotherapy of Cancer (which I organized this past year) would place him in close contact with the foremost research and researchers in cancer immunology and immunotherapy, developing his intellectual and collaborative skill directly as it relates to cancer (scientifically and clinically) and cancer immunotherapy.

Clinical Experiences:

Jeff will be working in numerous clinical capacities during the training period, which will help develop the clinical skills necessary for a future career as a physician-scientist with direct patient and clinical trial involvement. The Longitudinal Clinical Clerkships (LCCs), which represent 20 week clinical rotations for one half-day per week, will be specifically focused on medical oncology, in concordance with Jeff's clinical and career interests. This will provide highly valuable exposure to clinical fields directly related to and impacted by his concurrent research, enhancing the research/clinical integration important to his planned career as a translational investigator. He has already completed one such LCC with Dr. Suzanne Lentzsch, MD, PhD (Assistant Professor of Medicine with the Division of Hematology/Oncology; Clinical Director of the Multiple Myeloma Program), for which he received outstanding performance evaluations. Dr. Lentzsch is also one of our research collaborators, providing a unique opportunity for Jeff to directly experience the integration between research and clinical practice. During this fellowship period, Jeff will complete another LCC with Dr. John Kirkwood, MD (Professor and Vice Chair for Clinical Research, Department of Medicine; Director, Melanoma Program; Chairman, Melanoma Committee of the Eastern Cooperative Oncology Group). Dr. Kirkwood is a highly accomplished researcher and clinician, as well as a close collaborator with our lab and a co-author on Jeff's most recent publication. He would provide the ideal mentor for integrating Jeff's research and clinical interests, and facilitate the development of his clinical cancer research skills.

This clinical experience during the research portion of the fellowship will subsequently transition to majority-time clinical work in completion of his medical degree, although his continued involvement in our lab's data analysis and critical review of lab manuscripts will maintain the connection to his thesis training. The relationship developed with Dr. Kirkwood during his LCC experience will also provide the foundation for a planned clinical research experience during Jeff's 3rd and 4th year of medical school, in which he will participate in discrete clinical research projects during an elective research rotation. This will allow him to gain a better understanding of research from a clinically-focused perspective, as a supplement to the laboratory research experience gained from his thesis work.

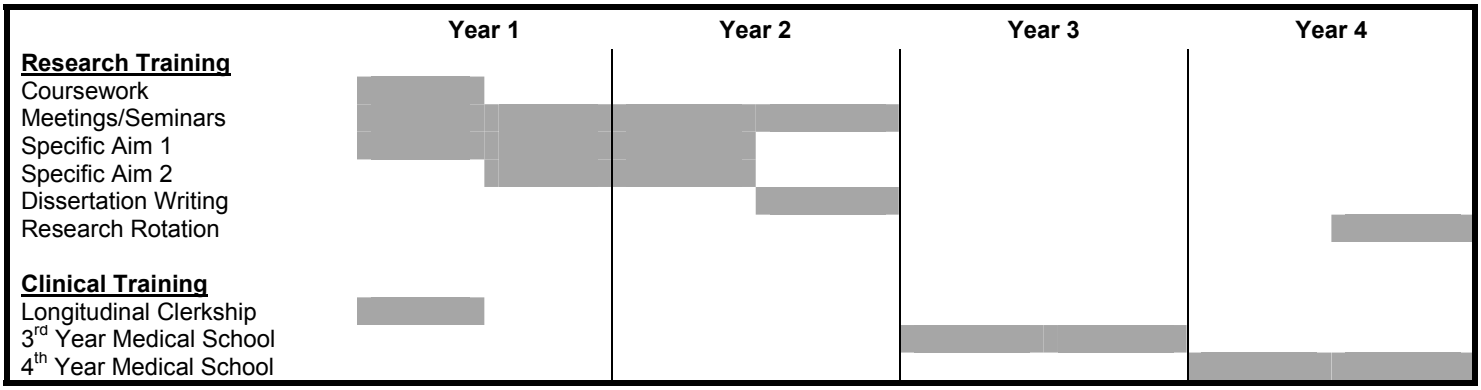
Professional Development:

In addition to the already-completed MSTP Professional Development course, which provided formal instruction on scientific writing, presenting, and networking, Jeff will participate in various activities designed specifically to enhance his professional skills. His oral communication will be developed through his participation in the meetings and seminars described above, as well as regular presentation of his data, critique of other projects, and literature presentation within our weekly lab meetings. His written skills will be developed throughout the research and clinical training period via his personal manuscript preparation, with input from me and other lab members and collaborators, as well as through his critical review of manuscripts and grant applications prepared by others. Finally, mentorship, teaching, and leadership skills will be

developed through a formal teaching assistantship for the School of Medicine Medical Microbiology course.

Ethics Training:

Training in the responsible conduct of research and clinical care is a key aspect of the MSTP dual-degree program at the University of Pittsburgh. This training comprises multiple formats integrated longitudinally across both medical and graduate phases. Jeff will have already taken a semester-long didactic/workshop course on Ethics, Law, and Professionalism provided as part of the medical school curriculum as well as an additional month-long didactic/workshop course on Ethics for Medical Scientists through the MSTP program. As relevant to Jeff's work, he has also completed self-directed internet-based training modules on research integrity, human subject research in biomedical science, conflict of interest, and HIPAA privacy requirements for researchers. Jeff further participates in ethics-based seminars twice a year through the MSTP program, and will continue to participate in these seminars for the duration of his training. Please see the section on the "Responsible Conduct of Research" for a detailed description of all of these activities.



II.C.2. Environment

Kalinski Laboratory:

My laboratory is highly-experienced and well-suited for training independent translational investigators with clinically-relevant roles, as evidenced by my past trainees (see section II.B), which include several MD/PhDs. Additionally, as a member of the Interdisciplinary Biomedical Graduate Program of the School of Medicine and of the Graduate Program of the Graduate School of Public Health, I am or have been a member of eight comprehensive exam committees and a member of nine PhD thesis committees. I am also a member of the Junior Faculty Mentoring Committee of the UPCI, developed in order to support the transition of our junior faculty members to tenure-track positions. I also currently serve as a Co-Investigator on a T32 training grant specifically focused on training fellows in research relevant to oncology and the biological therapy of cancer.

Jeff will benefit from this experience during regularly scheduled, one-on-one weekly meetings with me to discuss the progress of his projects, abstract and manuscript preparation, and other career and professional development issues (see "Training Plan" section II.C.1 for more detail). Lab members can further consult with me at any time on an ad hoc basis (my office is adjacent to the lab). The training potential of my group is enhanced by the availability of a senior group member and research manager, Dr. Eva Wieckowski, with vast experience in the field of cancer immunology and immunotherapy. My research group also benefits from a faculty Research Instructor, Dr. Ravikumar Muthuswamy, with considerable experience in tumor immunology, especially as it relates to clinical tumor materials (directly relevant to Jeff's proposal). The lab training environment is also supported by two postdoctoral fellows with considerable experience in imaging and other immunologic assays, including as they specifically relate to myeloid-derived suppressor cells from ovarian cancer (also directly relevant to his proposal). Jeff may also benefit from practical advice and an exchange of ideas with a senior graduate student in the lab. All lab members also regularly present their projects to the entire group (every 2 months) during our weekly group meetings, receiving valuable scientific feedback.

University of Pittsburgh, Division of Surgical Oncology of the University of Pittsburgh Cancer Institute (UPCI), and the Magee-Womens Hospital (MWH)/Magee-Womens Research Institute (MWRI):

The research and training potential of my lab is enhanced by ongoing interactions within the extremely rich scientific and training context of the University of Pittsburgh, the UPCI, and the MWH/MWRI.

The University of Pittsburgh is widely recognized as a leading academic institution in the US. Consistently within the top 10 recipients of overall NIH funding, the focus of the university's research efforts on medical science and biotechnology places our group in the center of the university's research and training activities.

The commitment of the university to the research and career development of pre-doctoral MD/PhD trainees is emphasized by the strength of the MSTP, which enjoys strong funding support from both the NIH and the School of Medicine and the resources necessary to develop and support a unique, highly-integrated curricular training program tailored specifically for combined medical and graduate research training.

The University of Pittsburgh Cancer Institute (UPCI) is the only NCI-designated Comprehensive Cancer Center in western Pennsylvania. In 2010, the UPCI received almost $170 million in research funding and was ranked 11th nationally in funding from the NCI, demonstrating its strength in biomedical cancer research and the quality of the colleagues and mentors Jeff will have access to during his training. The UPCI is housed in the Hillman Cancer Center, which brings under one roof diverse clinical, research, and educational/training facilities. This will facilitate Jeff's interactions with other research groups, networking with both research and clinical faculty, and acquisition of clinical materials for his studies. The large number of cancer-oriented scientists, research programs, and visiting scientists results in a high number of research seminars and conferences related to cancer, which Jeff has access to, as well as an extremely robust UPCI annual retreat.

I serve as the Direct of Research for the Division of Surgical Oncology within the UPCI. Intensive interactions between the scientific and clinical faculty of the Division facilitates joint development and clinical introduction of new cancer therapies, with a specific focus on cancer immunotherapy. Jeff will directly benefit from being situated within this Division through exposure to this translational cancer research integration. Jeff will also benefit from our close association with Magee-Womens Hospital (MWH), recognized as a National Center of Excellence in Women's Health and among the top 12 hospitals nationwide for gynecological care, and the Magee-Womens Research Institute (MWRI), the nation's first research center devoted exclusively to health conditions affecting women and their infants. Particularly relevant to Jeff's proposal is the strength of MWH's Division of Gynecologic Oncology, which sees over 100 newly-diagnosed ovarian cancers yearly. Facilitated through his interactions with Dr. Robert Edwards, MD (Director of the Ovarian Cancer Center of Excellence, our close research collaborator, and a member of Jeff's thesis committee), Jeff will have access to the considerable expertise of this division and to patient materials necessary for the completion of his proposal.

II.C.3. Research Facilities and Equipment

My lab occupies approximately 1,200 sq. ft. of space on the first floor of the Hillman Cancer Center Building. The lab is fully equipped for cellular immunology research, with: 4 laminar flow hoods, 4 CO_2 incubators, 3 adjustable speed/temperature cell centrifuges, 3 inverted microscopes, a PCR cycler (Taqman), water bath incubators, a histologic microscope, 2 Eppendorf centrifuges, 3 scales, 2 -80°C freezers, 2 -20°C freezers, 2 -4°C refrigerators, other minor laboratory equipment, and the necessary software and hardware for data analysis. Shared facilities in immediate proximity provide equipment for ELISA and ELISPOT analysis, liquid nitrogen cryopreservation tanks, gamma and beta counters, HPLC equipment, histology cryostats, cell harvesters, and high speed- and ultra-centrifuges. Space and fixtures include an additional molecular biology room with fume hood, molecular histopathology room, dark room, and walk-in cold room. As a part of the UPCI, Jeff will have further full access to its extensive core facilities (the most relevant below):

- Flow and Imaging Cytometry Core (adjacent to the lab): contains equipment for 4- and 12-color flow cytometry, cell-sorting, and a Cellomics Arrayscan HCS reader (Thermo Scientific)
- Biologic Imaging Satellite Facility (adjacent to the lab; led by Dr. Per Basse, MD, PhD, a member of Jeff's thesis committee): contains multiple light microscopes, a temperature- and CO_2-controlled time-lapse imaging microscope, confocal and two-photon microscopes, and microtomes
- Luminex Core: provides a Luminex Bio-Plex workstation for cytokine/chemokine profile analysis
- Tissue and Research Pathology Services: provides centralized tissue and biological specimen procurement, research histology, annotated clinical data, and tissue microarray services

Other core facilities include a Viral Vector Core, Peptide Synthesis Facility, Proteomics and Mass Spec Core, Immunologic Monitoring Facility, and Biostatistics Core (see "Equipment" section). Jeff will have further access to the Center for Biologic Imaging, which encompasses 17 confocal microscopes, 2 TEM microscopes, 1 SEM microscope, 17 additional assorted microscopes for various applications, extensive imaging processing and analysis hardware and software, and biologic imaging expertise of dedicated staff. Additional intellectual resources include clinical research expertise through the Clinical and Translational Science Institute.

II.D. Number of Fellows/Trainees to be Supervised

I am currently supervising two graduate students and two postdoctoral fellows.

II.E. Applicant's Qualifications and Potential for a Research Career

As an investigator with a career-long involvement in translational cancer research, I have come to appreciate unique qualities necessary for success in the demanding research environment at the junction

between the lab and the clinic. These qualities include intellectual curiosity, dedication, and originality, but also the practical insight, collaborative skill, and communication necessary to translate scientific knowledge into practical application. Having had the opportunity to mentor several clinical-oriented research trainees in my career, I know these qualities are actively embodied and cultivated only by certain driven individuals. I believe Jeff is one such individual, and I believe that, with the help of this fellowship, he has the outstanding potential to develop into a highly successful, independent investigator focused on translational cancer research.

I have known Jeff since spring 2008, when he applied for a summer rotation in my lab. I accepted him as a rotation student being intrigued by both his impressive CV (already at that stage he was a co-author of several papers and the first author of several research abstracts) and by his intellectual curiosity for how the immune system works and how it can be modulated to help patients with cancer. Over the summer of 2008 and since the beginning of his PhD work in the summer of 2009, I have had ample opportunity to evaluate his research ability and potential for becoming an independent researcher. From the very first weeks of our interaction, I was impressed with his scientific creativity and enthusiasm and his ability to formulate hypotheses, design experiments, perform them, and interpret the data. He also displayed impressive initiative in advancing collaborative studies, understanding the value of joining expertise toward a common question. For instance, he worked with the other graduate student in the lab to develop parallel studies comparing natural killer and cytotoxic T cell interactions with dendritic cells and tumor cells. This has also translated to his interactions with others outside of our lab. For instance, he has developed an arm of his proposal through discussions with another mentor, Dr. Michael Lotze, to combine our expertise in NK and DC biology with their expertise in cell death mechanisms to investigate how different modes of NK cell activation may affect anti-tumor responses.

Of particular interest to me was (and continues to be) Jeff's interest in NK cell immune-regulatory interactions and the translational therapeutic significance of these findings for patients with cancer. His dedication to becoming an expert in this field is unmistakable, manifested in his continued rigorous survey of the available scientific literature. This is supported by his incredible work ethic, his practical problem-solving abilities when obstacles arise, and his very receptive use of guidance and mentorship from multiple sources. These attributes have contributed to a very productive start to his PhD, highlighted by a first author manuscript and presentation of his work at international symposia. He has also prepared a strong thesis proposal, which he successfully defended as part of the Immunology Department's Comprehensive Examination more than half a year earlier than other students in his same PhD matriculating class. I was extremely impressed by the research proposals Jeff independently prepared for his thesis and this application, which indicates his exceptional potential for original research thought. Jeff has also demonstrated the key ability to be able to communicate not only his scientific findings, but also the scientific and clinical significance of his work, which is vital to a successful career in translational research. For instance, he was recently selected as a Richard L. Simmons Award recipient as a top oral presenter in the Department of Surgery Annual Research Symposium, making his work relevant to a challenging audience of diverse scientific and clinical backgrounds.

Taking into account Jeff's scientific interests, his impressive past experience with several laboratories (including with the NIH), and his documented record of success (including among many others, as College of Liberal Arts and Sciences Valedictorian at the University of Florida and selection for the national Goldwater and Beckman Fellowships, an NCI Cancer Research Training Award, and an Individual Pre-Doctoral Fellowship from the Clinical and Translational Science Institute based on proposals of his own design), I have very high expectations for his future success as a physician-scientist. I believe that his scientific interests and his career goals are highly compatible with my research group, which translates findings on NK cell and CTL interactions with dendritic cells to develop new cancer immunotherapies. Since several of these approaches involve close collaboration with physicians and physician-scientists, they will provide the patient samples relevant to his proposal and the opportunity for Jeff to be exposed to the logistic and regulatory issues related to the conduct of clinical studies. Already, Jeff has fully utilized these collaborations to refine his scientific and clinical interests. For instance, he initiated a Hematology/Oncology clinical clerkship with our collaborators, Dr. Suzanne Lentzsch, MD, PhD (Clinical Director of the Multiple Myeloma Program) and Dr. Markus Mapara, MD, PhD (Director of the Hematopoietic Stem Cell Transplantation Program), for which he earned outstanding 'honors' evaluations for his clinical performance while simultaneously maintaining his lab productivity and developing some very interesting ideas for future collaborative studies. This truly demonstrates Jeff's capability and potential for synergistically integrating his research and clinical pursuits.

Based on the particularly strong record of Jeff's past achievements and my own personal experience as his research supervisor, I am looking forward to continuing my role as Jeff's mentor, and believe that with the help of the currently described training, he will become an exceptional physician-scientist set for an outstanding career in translational cancer research.

OMB Number:
Expiration Date:

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: | * First Name: Jeffrey | Middle Name: Ling-Yi
* Last Name: Wong | Suffix:
Position/Title: MD/PhD Candidate | Department: Surgery
Organization Name: University of Pittsburgh | Division: Surgical Oncology
* Street1: 5117 Centre Ave
Street2:
* City: Pittsburgh | County/ Parish: Allegheny
* State: PA: Pennsylvania | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 15213-1863
* Phone Number: 412-623-3251 | Fax Number: 412-623-7709
* E-Mail: wong.jeffrey@medstudent.pitt.edu

Credential, e.g., agency login: wongjl

*** Project Role:** PD/PI | **Other Project Role Category:**

Degree Type: BS;BA
Degree Year: 2007

***Attach Biographical Sketch** Wong_Biosketch_JLW_F30.pdf [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 1

Prefix: | * First Name: Pawel | Middle Name:
* Last Name: Kalinski | Suffix:
Position/Title: Professor | Department: Surgery
Organization Name: University of Pittsburgh | Division: Surgical Oncology
* Street1: 5117 Centre Ave
Street2:
* City: Pittsburgh | County/ Parish: Allegheny
* State: PA: Pennsylvania | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 15213-1863
* Phone Number: 412-623-7712 | Fax Number: 412-623-7709
* E-Mail: kalinskip@upmc.edu

Credential, e.g., agency login: pkalinski

*** Project Role:** Other (Specify) | **Other Project Role Category:** Sponsor

Degree Type: MD;PhD
Degree Year: 1991;1998

***Attach Biographical Sketch** Kalinski_Biosketch_JLW_F30.pd [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

FELLOWSHIP APPLICANT BIOGRAPHICAL SKETCH

USE ONLY FOR INDIVIDUAL PREDOCTORAL and POSTDOCTORAL FELLOWSHIPS. DO NOT EXCEED FOUR PAGES.

NAME OF FELLOWSHIP APPLICANT	POSITION TITLE
Jeffrey L. Wong	M.D./Ph.D Candidate
eRA COMMONS USER NAME (credential, e.g., agency login) wongjl	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Florida, Gainesville, FL	B.S./B.A.	2007	Microbiol/Biochem/Econ
University of Pittsburgh, Pittsburgh, PA	Ph.D	2013	Immunology
University of Pittsburgh, Pittsburgh, PA	M.D.	2015	Medicine

A. Personal Statement

My ultimate career goal is to become an active physician-scientist at the direct interface between rigorous science and evidence-driven clinical innovation. Within a large research institution and an adjunct trial-oriented academic hospital, I plan on translating *in vitro, in vivo,* and *ex vivo* bench findings on clinically-relevant patient materials to therapeutic clinical trials, which I will directly develop and oversee. Tumor immunology and immunotherapy are of particular interest to me, both scientifically and clinically. My current PhD thesis work focuses on the immunologic interactions between natural killer cells and other key cells (including dendritic cells and myeloid-derived suppressor cells) in the human tumor context and their implications for cell-based cancer therapies, training which will provide a valuable scientific, technical, and professional framework for future independent scientific work in this highly translational field. I plan on further pursing my MD with a specialization in medical oncology. I hope to use these clinical skills to establish a specialized clinical role from which I will gain first-hand knowledge of the medical needs I will target in my research, as well as provide a source for patient enrollment in therapeutic trials. I believe my past research experiences and accomplishments provide a solid foundation for these pursuits, including multidisciplinary work resulting in several authorships and abstracts; experience at the National Cancer Institute under Dr. Steve Rosenberg, an internationally-recognized leader in cancer immunotherapy; numerous medical school and undergraduate academic recognitions; and competitive selection for multiple national and institutional fellowships based on original research proposals. Combined with the training and mentorship plan outlined in the proposal, I believe this fellowship would be instrumental in developing the scientific, technical, regulatory, and ethical skills necessary for a future role as an independent physician-scientist investigator in translational cancer research.

B. Positions and Honors

ACTIVITY/OCCUPATION	BEGINNING DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/COMPANY	SUPERVISOR/ EMPLOYER
Research Assistant	6/02	6/03	Molecular Biology	Altor Bioscience	Dr. Shari Schiavi/ Dr. Heather Belmont
Undergraduate Researcher	5/04	5/06	Chemistry	University of Florida	Dr. Randolph Duran
Research Assistant	6/06	8/06	Immunology	National Cancer Institute	Dr. Richard Morgan/ Dr. Steven Rosenberg
MD/PhD Candidate	6/07	Present	Clinical Medicine/ Immunology	University of Pittsburgh	Dr. Clayton Wiley (MSTP Director)

Academic and Professional Honors:

National Merit Scholarship, 2003-2007
John V. Lombardi Scholarship, Univ of Florida, 2003-2007

1 of 8; top university-wide award providing substantial support for students with outstanding academic, service, leadership, and research achievement

Wentworth Scholar, Univ of Florida, 2004
1 of 25; university-wide academic honor recognizing outstanding sophomores

Presidential Award for Undergraduate, Graduate, and Professional Students, Univ of Florida, 2005
University-wide academic, research, leadership, and service honor

Anderson Scholar, Univ of Florida, 2005
University-wide academic honor recognizing outstanding juniors

Who's Who Among Students in American Universities and Colleges, Univ of Florida, 2005
1 of 20; university-wide academic, research, leadership, and service honor

College of Agriculture and Life Sciences Grebe-Wahlberg Scholarship, Univ of Florida, 2005
1 of 3; college-wide academic honor

College of Agriculture and Life Sciences Scholarship, Univ of Florida, 2006
1 of 2; college-wide academic honor

University of Florida Outstanding Scholar, Univ of Florida, 2007
1 of 10; university-wide honor recognizing outstanding academics, research, leadership, and service

College of Liberal Arts and Sciences Valedictorian, Univ of Florida, 2007
Top student in 2007 graduating class

Phi Beta Kappa, Univ of Florida, 2007

Phi Kappa Phi, Univ of Florida, 2007

B.S./B.A. with majors in Microbiology and Cell Science, Chemistry-Biochemistry, and Economics
Awarded summa cum laude, Univ of Florida, 2007

Dean's Merit Scholarship, Univ of Pittsburgh School of Medicine, 2007-2011
Academic and extracurricular achievement honor providing four years of scholarship support

Mentor, Univ of Pittsburgh School of Medicine Peer Academic Mentoring Program, 2008-2009
Top academic medical students chosen to mentor underclassmen

Grant Funding:

Beckman Fellowship, Arnold and Mabel Beckman Foundation, 2004-2006
1 of 22 nationwide; nationally-competitive individual fellowship awarded on academic merit, research potential, and an original research proposal in chemistry or biology; provides two years of support for scholarship and completion of an original research proposal

Cancer Research Training Award, National Cancer Institute, National Institutes of Health, 2006
Individual fellowship award supporting a research project in cancer under an intramural National Cancer Institute investigator

Barry M. Goldwater Fellowship, Barry M. Goldwater Scholarship Foundation, 2006-2007
1 of 323 nationwide; nationally-competitive individual fellowship awarded on academic achievement, research excellence, and an original research proposal in the natural sciences, mathematics, or engineering; provides two years of support for scholarship and completion of an original research proposal

Medical Scientist Training Program Fellowship, National Institutes of Health/Univ of Pittsburgh, 2007-2008
Grant No. T32 GM008208

Individual Predoctoral Fellowship, Clinical and Translational Science Institute, National Institutes of Health/Univ of Pittsburgh, 2010-2011
Grant No. TL1 RR024155; individual funding awarded on a competitive basis, chosen based on an original research proposal, training plan, and potential as an independent investigator

Memberships in professional societies:

American Medical Association

C. Publications

Research papers:

Belmont HJ, Schiavi S, Liu B, Card KF, Lee H, Han K, Wen J, Tang S, Zhu X, Merrill J, Chavillaz P, **Wong JL**,

Rhode PR, Wong, HC. Potent antitumor activity of a tumor specific soluble TCR/IL-2 fusion protein. Clin Immunol, 2006, 121, 29-39.

Chavez JL, **Wong JL**, Duran RS. Core-shell nanoparticles: Characterization and study of their use for the encapsulation of hydrophobic fluorescent dyes. Langmuir, 2008, 24, 2064-2071.

Wen J, Zhu X, Liu B, You L, Kong L, Lee HI, Han KP, **Wong JL**, Rhode PR, Wong HC. Targeting activity of a TCR/IL-2 fusion protein against established tumors. Cancer Immunol Immunother, 2008, 57, 1781-1794.

Jiao JA, Kelly AB, Marzec UM, Nieves E, Acevedo J, Burkhardt M, Edwards A, Zhu XY, Chavaillaz PA, Wong A, **Wong JL**, Egan JO, Taylor D, Rhode PR, Wong HC. Inhibition of acute vascular thrombosis in chimpanzees by an anti-human tissue factor antibody targeting the factor X binding site. Thromb Haemost, 2010, 103, 224-233.

Wong JL, Mailliard RB, Moschos SJ, Edington H, Lotze MT, Kirkwood JM, Kalinski P. Helper activity of natural killer cells during the dendritic cell-mediated induction of melanoma-specific cytotoxic T cells. J Immunother, 2011, 34, 270-278.

Reviews

Chavez JL, **Wong JL**, Jovanovic AV, Sinner EK, Duran RS. Encapsulation in sub-micron species: A short review and alternate strategy for dye encapsulation. IEE Proc-Nanobiotechnol, 2005, 152, 2, 73-84.

Selected Abstracts

Wong JL, Chavez JL, Duran RS. Silica core-shell nanoparticles. Abstract for oral presentation, University of Florida Presidential Inauguration Academic Symposium (Oct 2004)

Wong JL, Chavez JL, Duran RS. Fluorescence behavior of dansyl chloride in polysiloxane/silicate core-shell nanoparticles. Abstract for poster presentation, 229th American Chemical Society National Meeting (Mar 2005)

Wong JL, Chavez JL, Duran RS. Core-shell nanoparticles as scavengers for hydrophobic molecules in biological systems. Abstract for oral presentation, 72nd Annual Meeting of the Southeastern Section of the American Physical Society (Nov 2005)

Wong JL, Chavez JL, Joncheray TJ, Duran RS. Design and characterization of polysiloxane/silicate core-shell nanoparticles for dynamic uptake and delivery systems. Abstract for poster presentation, 231st American Chemical Society National Meeting (Mar 2006)

Wong JL, Kim H, Zhu X, Wong HC, Ferris RL. Processing and presentation of HLA-A2-p53 complexes by HPV-infected cancers: Implications for p53 based immunotherapy. Abstract for poster presentation, American Medical Association Fall 2008 National Meeting (Nov 2008)

Wong JL, Giermasz A, Mailliard RB, Muthuswamy R, Wieckowski E, Lotze MT, Kalinski P. Potent induction of NK cell tumoricidal functions by type-1-polarized DC: Positive feedback between DC and NK cells. Abstract for poster presentation, International Society for Biological Therapy of Cancer 24th Annual Meeting (Oct 2009)

Wong JL, Mailliard RB, Kirkwood JM, Kalinski P. Helper activity of NK cells for the enhancement of dendritic cell-based cancer vaccines. Abstract for oral presentation, Richard L. Simmons Award for Top Oral Presentation, Univ of Pittsburgh Dept of Surgery Annual Research Symposium (May 2010)

Wong JL, Mailliard RB, Moschos SJ, Edington H, Lotze MT, Kirkwood JM, Kalinski P. Helper activity of NK cells during the dendritic cell-mediated induction of melanoma-specific CTL responses. Abstract for poster presentation, International Society for Biological Therapy of Cancer 25th Annual Meeting (Oct 2010)

D. Scholastic Performance

SCIENCE			OTHER		
YEAR	COURSE TITLE	GRADE	YEAR	COURSE TITLE	GRADE
	Univ of Florida			Univ of Florida	
2003	Organic Chemistry I		2003	Calculus 3 Honors	

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/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	POSITION TITLE
Pawel Kalinski MD PhD	Professor of Surgery, Immunology, and Infectious Diseases and Microbiology; Director of Research, Division of Surgical Oncology
eRA COMMONS USER NAME (credential, e.g., agency login) pkalinski	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Warsaw Medical University (AMW)	MD	10/1984-06/1991	Medicine
University of Amsterdam	fellowship	07/1991-06/1992	Immunology
Ctr. Clin. Hosp. Milit. Sch. Med, Warsaw	residency	08/1992-02/1994	Medicine
University of Amsterdam	PhD	02/1994-10/1998	Immunology
University of Amsterdam	fellowship	10/1998-02/2000	Immunology

A. Personal Statement

I believe I am highly qualified to sponsor this applicant for the NRSA F30 Fellowship. I have extensive past experience in training translational investigators (20 prior or current trainees, including several MD/PhDs, many with tenure or tenure-track appointments) for future careers in independent cancer research. Additionally, as a member of the Interdisciplinary Biomedical Graduate Program of the School of Medicine and of the Graduate Program of the Graduate School of Public Health, I am or have been a member of eight comprehensive exam committees and a member of nine PhD thesis committees. I am also a member of the Junior Faculty Mentoring Committee of the UPCI, developed in order to support the transition of our junior faculty members to tenure-track positions. I also currently serve as a Co-Investigator on a T32 training grant specifically focused on training fellows in research relevant to oncology and the biological therapy of cancer. The applicant will fully benefit from this scientific and professional mentorship experience, as outlined in the fellowship training plan.

The goal of my research is to understand the interactions of cancer with the immune system and to develop effective cancer immunotherapies involving the selective induction of tumor-specific CTLs, Th1 cells, and activated NK cells capable of homing to tumors while avoiding suppressive mechanisms associated with advanced cancer (such as hyper-activation and accumulation of MDSCs and Tregs in tumors). My lab has extensive expertise in dendritic cell vaccines, including in the use of NK cell helper activity to polarize DC vaccines for desirable anti-cancer type-1 immune responses. The applicant's proposed research falls directly within my research interests and expertise, and thus will fully benefit from my scientific and professional experience and relationships in the field. His interest in an eventual career in directly taking cancer immunotherapies from the bench to clinical care is also highly compatible with myself and my group. Our work has resulted in the development and implementation of several clinical trials in colorectal cancer, melanoma, and prostate cancer, with additional trials in ovarian cancer, colon cancer, melanoma, and CLL awaiting implementation, providing the applicant with excellent exposure to true translational research and experience with research involving clinical cancer materials for his future career in translational cancer research.

B. Positions and Honors

Professional Training and Experience

1986-1991	Research/Teaching Assistant Part-Time, Department of Immunology, Warsaw Sch. Med.
1991-1992	Fellow, European Community TEMPUS Program, University of Amsterdam
1992-1994	Assistant, Dept. Immunol., Central Clin. Hosp., Military School of Medicine, Warsaw, Poland
1994-1998	Assistant in Training, Department Cell Biology and Histology, University of Amsterdam
1998-2000	Postdoctoral Fellow, Department Cell Biology and Histology, University of Amsterdam
2000 - 2007	Assistant Professor of Surgery, Department of Surgery University of Pittsburgh
2001 - present	Member, University of Pittsburgh Cancer Institute, University of Pittsburgh
2002 - 2007	Assistant Professor of Immunology. University of Pittsburgh School of Medicine
2003 - 2007	Assistant Professor of Infectious Diseases and Microbiology, University of Pittsburgh
2006 - present	Member, McGowan Institute of Regenerative Medicine, University of Pittsburgh

2006 - present	Director of Research, Division of Surgical Oncology
2007 - 2010	Associate Professor of Surgery, University of Pittsburgh (tenured)
2007 - 2010	Associate Professor of Immunology, University of Pittsburgh
2007 - 2010	Associate Professor of Infectious Diseases and Microbiology, University of Pittsburgh
2010-present	Professor of Surgery, Immunology, & Inf. Dis. Microbiol., University of Pittsburgh (tenured)
2010-present	Director, Immunotransplantation Center, Surgical Oncology, UPCI
2010-present:	Member, Research Executive Advisory Committee of the UPCI

Professional Memberships

1993	International Society for Experimental Hematology
1993	Polish Society for Immunology
1995	The Netherlands Society for Immunology
2001	American Association of Immunologists
2004	Society for Natural Immunity
2008	International Society for Biological Therapy of Cancer

Honors

1989	Award of the Polish Medical Society
1993	Award of the Polish Science Foundation
1993	New Investigator Award of the International Society for Experimental Hematology
1998	"Cum Laude" (awarded to less than 5% of PhD in The Netherlands)
2000	Kathy Salling Memorial Award (of the Melanoma Research Foundation)
2001	Pittsburgh Foundation Awards
2005	Pittsburgh Foundation Awards
Since 2000:	*Ad hoc* reviewer for six NIH review panels and 20 additional research-funding agencies
2009-2010:	(co)Organizer 25th annual Meeting of the International Society of the Biologic Therapy of Cancer (iSBTc 2010).
2010-2011:	Organizer (Chair) of the 14th annual Meeting of the Translational Regional Consortium of Cancer Centers (TRC3 2011).

Editorial

2006-2009	Associate Editor, the Journal of Immunology
2009-2011	Section Editor, the Journal of Immunology
2011-2013	Section Editor, the Journal of Immunology (second term)

C. 15 Selected Peer-reviewed Publications (of 85 publications; over 4,000 citations)

1. Lasek W, Jakobisiak M, Grochowska M, Gorecki D, Gniadecki R, Kalinski P. (1988). The influence of pretransplant and posttransplant immunosuppression on cardiac graft survival in the donor-specific transfusion model in mice. Comparison of the effects of Cyclophosphamide, Procarbazine, Cyclosporine, and Cortisone. *Transplantation 47: 913-915.* PMID: 2655232.
2. Kalinski P, Schuitemaker JH, Hilkens CM, Kapsenberg ML. (1998). Prostaglandin E_2 Induces the Final Maturation of IL-12-Deficient $CD1a^+CD83^+$ Dendritic Cells: The Levels of IL-12 Are Determined During the Final Dendritic Cell Maturation and Are Resistant to Further Modulation. *J. Immunol. 161: 2804.* PMID: 9743339.
3. Kalinski P, Schuitemaker JH, Hilkens CM, Wierenga EA, Kapsenberg ML. (1998). Final maturation of dendritic cells is associated with impaired responsiveness to IFNγ and with resistance to bacterial IL-12 inducers. Decreased ability of mature DC to produce IL-12 during the interaction with Th cells. *J. Immunol. 162: 3231.* PMID: 10092774
4. Vieira PL, de Jong E, Wierenga E, Kapsenberg M, Kalinski P. (2000). Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J. Immunol*, 164: 4507-4512. PMID: 10779751.
5. Kalinski P, Vieira PL, Schuitemaker JH, de Jong EC, Kapsenberg ML (2001). Prostaglandin E(2) is a selective inducer of interleukin-12p40 (IL-12p40) production and an inhibitor of bioactive IL-12p70 heterodimer. *Blood* 97, 3466-3469. PMID: 11369638
6. Mailliard RB, Egawa S, Cai Q, Wankowicz-Kalinska A, Bykovskaya SN, Lotze MT, Kapsenberg ML, Storkus WJ, and Kalinski P. (2002). Complementary Dendritic Cell-activating Function of $CD8^+$ and $CF4^+$ T

Cells: Helper Role of $CD8^+$ T Cells in the Development of T Helper Type 1 Responses. *J. Exp. Med. 195: 473-483.* PMID: 11854360
7. Mailliard RB, Wankowicz-Kalinska A, Cai Q, Wesa A, Kapsenberg ML, Kirkwood JM, Storkus WJ, and Kalinski P. (2004). Alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. *Cancer Res 64: 5934-5937.* PMID: 15342370.
8. Mailliard RB, Alber SM, Shen H, Watkins SC, Kirkwood JM, Herberman RB, and Kalinski P. (2005). IL-18-induced $CD83^+$ $CCR7^+$ NK Helper Cells. *J. Exp. Med*., *202:941-953.* PMID: 16203865.
9. Watchmaker P, Urban JA, Nakamura Y, Mailliard RB, Giermasz AS, Watkins SC, van Ham SM, and Kalinski P. (2008). Memory $CD8^+$ T Cells Protect DC from CTL Killing. *J. Immunol., 180:3857-3865.* PMID: 18322193 (PMCID N/A yet)
10. Muthuswamy R, Urban J, Lee JJ, Reinhart TA, Bartlett D, and Kalinski P. (2008) Ability of Mature Dendritic Cells to Interact with Regulatory T Cells Is Imprinted During Maturation. *Cancer Res.* 68: 5972-8. PMID: 18632653 (PMCID N/A yet)
11. Giermasz AS, Urban JA, Nakamura Y, Watchmaker P, Cumberland RL, Gooding W and Kalinski P. (2009). Type-1 polarized dendritic cells primed for high IL-12 production show enhanced activity as cancer vaccines. *Cancer Immunol. Immunother.,* 58(8):1329-36. PMID: 19156413. (PMCID N/A yet)
12. Watchmaker PB, Berk E, Muthuswamy R, Mailliard RB, Urban JA, Kirkwood JM, and Kalinski P. (2010). Independent Regulation of Chemokine Responsiveness and Cytolytic Function versus $CD8^+$ T Cell Expansion by Dendritic Cells. *J. Immunol.* 2010; 184: 591-597 PMID: 20018619 (PMCID N/A yet)
13. Muthuswamy, R., J. Mueller-Berghaus, U. Haberkorn, T.A. Reinhart, D. Schadendorf, and P. Kalinski. (2010). PGE_2 Transiently Enhances DC Expression of CCR7 but Inhibits the Ability of DCs to Produce CCL19 and Attract Naïve T Cells *Blood.* 116(9):1454-9. PMID: 20498301.
14. Okada, H., P. Kalinski, R. Ueda, A. Hoji, G. Kohanbash, T. E. Donegan, A. H. Mintz, J. A. Engh, D. L. Bartlett, C. K. Brown, H. Zeh, M. P. Holtzman, T. A. Reinhart, T. L. Whiteside, L. H. Butterfield, R. L. Hamilton, D. M. Potter, I. F. Pollack, A. M. Salazar, and F. S. Lieberman. (2010) Induction of CD8+ T-Cell Responses Against Novel Glioma-Associated Antigen Peptides and Clinical Activity by Vaccinations With {alpha}-Type 1 Polarized Dendritic Cells and Polyinosinic-Polycytidylic Acid Stabilized by Lysine and Carboxymethylcellulose in Patients With Recurrent Malignant Glioma. *J Clin Oncol*. E-Pub: Dec 13, 2010
15. Wong JL, Mailliard RB, Moschos SJ, Edington H, Lotze MT, Kirkwood JM, and Kalinski P. (2011). Helper activity of natural killer cells during the dendritic cell-mediated induction of melanoma-specific cytotoxic T cells. *J Immunother*, 34: 270-278 PMID: 21389871.

D. Research Support

Ongoing Research Support

P01 CA101944 **(Lotze/Kalinski)** **07/06/2005 – 06/30/2011**
(NIH/NCI)
"Integrating NK and DC into Cancer Immunotherapy"
Role: PI of Project 2

R01 CA095128 **(Kalinski)** **07/01/2010 – 06/30/2011**
(NIH/NCI)
"Regulation of DC Activity by Memory and Effector $CD8^+$ T cells"
Role: PI

P01 CA132714 **(Kalinski)** **04/27/2009 – 03/31/2014**
(NIH/NCI)
"Directing Tumor-specific T cells to Tumors"
Roles: Overall Program PI, PI of Project 1, PI of Core A, Co-I of Project 3

R01 CA134633 **(Ahrens/Kalinski: multi-PIs)** **05/10/2009 – 04/30/2012**
(NIH/NCI)
"Clinical Translation of 19F MRI to Visualize Cancer Immunotherapeutic Cells"
Role: PI (MPI)

P50 CA121973 **(Kirkwood/Falo)** **08/26/2008 – 07/31/2013**
(NIH/NCI)
"SPORE in Skin Cancer"
Role: PI of Project 3

T32 CA113263 **(Bartlett)** **07/01/2006 – 06/30/2011**
(NIH/NCI)
"Postdoctoral Research Training in Biotherapy of Cancer"
Role: Co-I

P01 CA109688 **(Whiteside)** **09/30/2006 – 07/31/2011**
(NIH/NCI)
"Immune Escape of Human Cancers: Cellular Mechanisms and Countermeasures"
Role: Co-I

Completed Research Support

PI (or Project Leader)

- R01 CA82016 (Kalinski) "Melanoma Associated T & DC Dysfunction and Death" NCI 2000-2006
- Melan. Res. Fnd. Award (Kalinski) "Application of IFN-γ polarized DC to induce anti-melanoma responses" Melan. Res. Fnd. 2001-2002
- Pittsburgh Fnd. (Kalinski) "DC1/EphA2-based cancer vaccines" Pgh.Fnd.2001-3
- R21 CA114931 (Kalinski) "Polarized DC as Melanoma Vaccine: Phase I Evaluation" NCI 2005–2009
- M2005-0021 (Kalinski) "αDC1-based Immunotherapy of Gastrointestinal Cancer" Pgh Fnd 2005-9
- DOD PC051118 (Kalinski) "DC1-Based Vaccine against Prostate Cancer" DOD 2005-2006

Co-I:

- P01 CA074343 (Finn) "Dendritic Cell Biology Biology and Therapy" NCI 2004-2009
- R01 CA087840 (Storkus)"Dendritic Cell Strategies to Elicit Tumor Reactive T Cells" NCI 2003-2008
- R01 CA63350 (Storkus) "Dendritic Cell-based Therapy of Mouse Tumors" NCI 2000-2006
- P01 EA055944 (Corb) "An Adjuvanted Therapeutic DNAVaccine for AIDS" NIAID 2003-2007
- P01 CA68067 (Storkus) "Cytokine Gene Therapy of Cancer" NCI 1999-2002
- P01 CA74343 (Lotze/Finn) "Dendritic Cell Biology and Therapy" NCI 1998-2002
- P01 CA 100327 (Storkus) "Cytokine Gene Therapy of Cancer Preclinical Studies" NCI 2005-2010

PHS Fellowship Supplemental Form

OMB Number:

A. Application Type:

From SF424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated here for your reference as you provide the responses that are appropriate for this Fellowship application.

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

B. Research Training Plan

1. Introduction to Application *(for RESUBMISSION applications only)*		Add Attachment	Delete Attachment	View Attachment
2. * Specific Aims	Specific_Aims_JLW_F30.pdf	Add Attachment	Delete Attachment	View Attachment
3. * Research Strategy	Research_Strategy_JLW_F30.pdf	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment

Human Subjects

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the involvement of human subjects, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Human Subjects Involved? ☒ Yes ☐ No

6. Human Subjects Involvement Indefinite?	☐ Yes	☒ No
7. Clinical Trial?	☐ Yes	☒ No
8. Agency-Defined Phase III Clinical Trial?	☐ Yes	☐ No

9. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
10. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
11. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
12. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Training Plan Sections

Please note. The following item is taken from the Research & Related Other Project Information form. The response provided on that page, regarding the use of vertebrate animals, is repeated here for your reference as you provide related responses for this Fellowship application. If you wish to change the answer to the item shown below, please do so on the Research & Related Other Project Information form; you will not be able to edit the response here.

Are Vertebrate Animals Used? ☐ Yes ☒ No

13. Vertebrate Animals Use Indefinite? ☐ Yes ☐ No

14. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
15. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
16. Resource Sharing Plan		Add Attachment	Delete Attachment	View Attachment
17. * Respective Contributions		Add Attachment	Delete Attachment	View Attachment
18. * Selection of Sponsor and Institution		Add Attachment	Delete Attachment	View Attachment
19. * Responsible Conduct of Research		Add Attachment	Delete Attachment	View Attachment

PHS Fellowship Supplemental Form

C. Additional Information

Human Embryonic Stem Cells

1. * Does the proposed project involve human embryonic stem cells? ☐ Yes ☒ No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s):

Fellowship Applicant

2. Alternate Phone Number: 9

3. Degree Sought During Proposed Award:

Degree:	If "other", please indicate degree type:	Expected Completion Date (month/year):	
OTH: Other	MD/PhD	05/2015	Reset Entry

4. * Field of Training for Current Proposal: 2200 IMMUNOLOGY

5. * Current Or Prior Kirschstein-NRSA Support? ☒ Yes ☐ No

If yes, please identify current and prior Kirschstein-NRSA support below:

* Level	* Type	Start Date *(if known)*	End Date *(if known)*	Grant Number *(if known)*	
Predoctoral	Institutional	09/01/2007	08/31/2008	2T32GM008208-19	Reset Entry
Predoctoral	Institutional	06/01/2010	05/31/2011	5TL1RR024155-04	Reset Entry
					Reset Entry
					Reset Entry

6. * Applications for Concurrent Support? ☐ Yes ☒ No

If yes, please describe in an attached file: | Add Attachment | Delete Attachment | View Attachment

7. * Goals for Fellowship Training and Career: Goals_for_Fellowship_Training_an | Add Attachment | Delete Attachment | View Attachment

8. * Activities Planned Under This Award: Activities_Planned_Under_This_Aw | Add Attachment | Delete Attachment | View Attachment

9. Doctoral Dissertation and Other Research Experience: Doctoral_Dissertation_Research_E | Add Attachment | Delete Attachment | View Attachment

10. * Citizenship:
☒ U.S. Citizen or noncitizen national
☐ Permanent Resident of U.S. Pending
☐ Permanent Resident of U.S.
(If a permanent resident of the U.S., a notarized statement must be provided by the time of award)
☐ Non-U.S. Citizen with temporary U.S. visa

PHS Fellowship Supplemental Form

C. Additional Information (continued)

Institution

11. ☐ Change of Sponsoring Institution

Name of Former Institution:

D. Sponsor(s) and Co-Sponsor(s)

* Sponsor(s) and Co-Sponsor(s) Information: Sponsor_Information_JLW_F30.pdf [Add Attachment] [Delete Attachment] [View Attachment]

E. Budget

All Fellowship Applicants:

1. * Tuition and Fees:

☐ None Requested ☒ Funds Requested:

Year 1	47,721.00
Year 2	50,107.00
Year 3	47,070.00
Year 4	49,424.00
Year 5	
Year 6 *(when applicable)*	
Total Funds Requested:	194,322.00

Senior Fellowship Applicants Only:

2. Present Institutional Base Salary: Amount | Academic Period | Number of Months [Reset Entry]

3. Stipends/Salary During First Year of Proposed Fellowship:

a. Federal Stipend Requested: Amount | Number of Months

b. Supplementation from other sources: Amount | Number of Months

Type (sabbatical leave, salary, etc.)

Source

F. Appendix

[Add Attachments] [Delete Attachments] [View Attachments]

Tracking Number:GRANT Funding Opportunity Number:PA-11-110 Received Date:2011-04-04T14:38:00-04:00

Section I – Research Training Plan

I.A. Specific Aims

While natural killer (NK) cells have been classically understood as innate cytotoxic effector cells, they have also been recently implicated as critical immune-modulators in the development of adaptive immunity. In particular, NK cell interactions with dendritic cells (DCs) have been shown to be instrumental in shaping effective anti-cancer immune responses, holding great implications for DC- and other cell-based cancer immunotherapies.

We have previously demonstrated that activated NK cells can exhibit desirable immune-stimulatory functions through distinct 'killer' and 'helper' activities, potentiating both spontaneous and vaccine-induced anti-tumor immune responses through direct tumor lysis or the enhancement of DC activation. However, our new preliminary data indicate that activated NK cells may also contribute to undesirable immune-suppressive functions through direct DC killing or the hyper-activation of myeloid-derived suppressor cells (MDSCs). Based on these observations, I **hypothesize that differentially-activated NK cells can selectively acquire and perform lytic and non-lytic immune-modulatory functions, which critically influence the anti-cancer outcome of their interactions with tumor cells, DCs, and MDSCs.** This project seeks to define the mechanisms underlying these differential NK cell functions to inform the therapeutic enhancement of NK- and DC-mediated anti-tumor activities. This project will pursue two Specific Aims:

I.A.1. Specific Aim 1: Define the mechanisms involved in 'killer' NK cell and 'helper' NK cell function during NK cell interaction with DCs and tumor cells. We have previously shown that exposure to IL-2 or IL-18 can activate resting human NK cells into unique 'killer' or 'helper' functional phenotypes, respectively. While IL-2-treated NK cells efficiently kill tumor cells and both immature and mature DCs, IL-18-treated NK cells instead induce DC maturation and the DC-mediated-polarization of Th1 and CTL responses. I will use this model of differential 'killer' and 'helper' activation to test the hypothesis that specific independently-regulated factors are selectively involved in the ability of NK cells to kill tumor cells and immature and mature DCs, as well as activate, rather than kill, DCs. This will provide critical knowledge in identifying prospective targets for the therapeutic uncoupling of the desirable DC-activating and tumor-killing functions of NK cells from the undesirable DC-killing functions of NK cells, enhancing productive NK-DC positive feedback for the improvement of cell-based cancer immunotherapies.

I.A.2. Specific Aim 2: Define the outcomes of activated NK cell interaction with MDSCs in the setting of human ovarian cancer (OvCa) and characterize the mechanisms governing these interactions. MDSCs are known to accumulate in most cancer patients and experimental animal models of cancer and play a critical role in tumor-associated immune-suppression, including the inhibition of DC-mediated T cell activation. Our preliminary data indicate that in contrast to their desirable type-1-polarizing interactions with DCs, activated IFNγ-producing NK cells may contribute to an undesirable hyper-activation of MDSC suppressive function. Using cells derived from MDSC-rich OvCa ascites as a clinically-relevant model, I will examine the effect of differentially-activated NK cells on MDSC suppressive functions and the effect of NK-mediated MDSC activation on DC immune-stimulatory capacity. Guided further by our preliminary data suggesting the essential role of sustained PGE_2 in maintaining MDSC functions, I will also assess whether inhibition of the COX2-PGE_2 axis may reverse the undesirable NK-mediated enhancement of MDSC activity, while preserving the desirable NK-mediated activation of DC immune-stimulatory function.

Overall, this study will provide critical new insights into the interactions between NK cells, DCs, and MDSCs, advancing our understanding of the immunologic responses fundamental to cancer. These studies will also inform the development of a single cohesive therapeutic strategy combining a 'preferred' mode of NK cell activation with rationally-selected pharmacologic or antibody-based functional modifiers to boost the desirable anti-cancer activities of NK cells (tumor-killing, MDSC-killing, and DC activation) while limiting their undesirable immune-suppressive activities (DC-killing and MDSC activation). The proposed research plan will also provide an ideal training vehicle for the development of the conceptual and technical scientific skills essential for a future career in independent translational cancer research.

I.B. Significance

I.B.1. Cancer immunotherapy and DCs. Cancer remains the leading cause of mortality under age 85 in the US despite advances in therapy and prevention[1]. While conventional treatment with surgery, radiotherapy, and chemotherapy can reduce tumor burden, such treatments are often ineffective in eliminating residual cancer, preventing disease recurrence, and extending patient survival, particularly in the setting of metastatic disease. Therapeutic modulation of the immune system to better recognize and control cancer progression could overcome these limitations[2]. Given their ability to efficiently prime naïve T cells to cancer antigen-specific and contextual effector function[3-5], including the instruction of effector cell homing to relevant transformed tissues[6], dendritic cells (DCs) in particular are attractive targets for cancer immunotherapy[7]. Combined with the additional ability of activated DCs to stimulate immunity beyond the T cell compartment[8], inducing diverse responses against potentially distinct, non-overlapping features of cancer alteration, this provides a strong rationale for DC-based cancer immunotherapy. Nevertheless, current success of DC-based therapies have been limited for most cancers[9,10], highlighting the critical need for further innovation in optimizing DC vaccines and their productive interactions with other immune cells to maximize their anti-cancer therapeutic potential.

I.B.2. Type-1-polarized DCs: Helper role of NK cells. Type-1 immune responses, dominated by the activation of IFNγ-producing $CD4^+$ T helper (Th1) cells, cytotoxic $CD8^+$ T cells (CTLs), and NK cells, are critical for developing the cellular immunity necessary for effective surveillance against tumor development. Differential polarization to type-1 responses is influenced significantly by DC-provided signals[4], with production of IL-12p70 serving as an essential factor[11]. Recent evidence suggests that NK cells, although originally characterized by their rapid cytotoxicity, play a critical 'helper' role in shaping innate and adaptive immune responses through their modulation of DC function[12]. Our group (including new preliminary data, see Fig 1) and others have demonstrated that NK cells, following recognition of MHC class I^{low} targets expressing ligands for NK activating receptors and/or exposure to various soluble mediators, including type I interferons, IL-2, and IL-18, can mediate DC activation via TNFα, IFNγ, and cell-to-cell contact-dependent signals, including NKp30[13-17]. Such 'helper' NK (NK_h) cells demonstrate the ability to promote high IL-12-producing type-1 polarized DCs with an enhanced capacity to induce anti-tumor Th1 and CTL responses, even in the absence of $CD4^+$ T cell help[18]. Understanding the mechanisms underlying the productive interactions between NK cells and DCs will help inform the rational use of these activated NK cells in generating more effective cancer vaccines.

I.B.3. NK cell killing of DCs. In addition to promoting and polarizing DC maturation, activated NK cells have also been shown to kill immature (i)DCs[14,19,20], acting as a potential suppressive pathway providing negative feedback control over immune activation. A number of mechanisms have been proposed to mediate this NK cell killing of iDCs, including iDC recognition via the NKp30, NKp46, and DNAM-1 activating receptors on NK cells[21-23], and execution through multiple potential pathways, including perforin, granzyme, FasL, and TRAIL-dependent mechanisms[19,24]. Although mature (m)DCs have been traditionally thought to resist NK cell killing due to the upregulation of MHC ligands for NK cell inhibitory receptors, our preliminary data demonstrates that IL-2-activated NK cells can efficiently lyse both iDCs and mDCs (Fig 1A), consistent with prior observations demonstrating efficient killing of LPS-activated macrophages despite upregulated MHC[25]. This NK cell killing of both iDCs and mDCs is in contrast to the selective perforin/granzyme B (GrB)-mediated killing only of iDCs by human CTLs, shown by our group to be due to mDC protection by the endogenous GrB inhibitor PI-9[26]. Coupled with our preliminary data indicating similar GrB/perforin levels between DC-killing NK_k cells and DC-activating NK_h cells (data not shown), this suggests a unique and previously unappreciated difference in the DC-killing mechanism by human NK cells compared to CTLs. Understanding and comparing the mechanisms of NK cell killing of DCs and tumor cells will be critical to defining strategies for the separation of the desirable tumor-killing and undesirable DC-killing functions of NK cells for the improvement of cell-based cancer therapy.

I.B.4. Differential activation of NK cell 'helper' (NK_h) and killer (NK_k) activity. Our prior observations[16,27] and our new preliminary data directly in the context of late stage cancer patients indicate that IL-18, while not promoting enhanced NK cell lytic capacity (Fig 1A), primes resting NK cells to a unique 'helper' phenotype (NK_h) that synergizes with additional signals, including type I interferons, to produce high levels of IFNγ (Fig 1B). These NK_h cells induce mature type-1-polarized DCs ($_{NK}$DC1s) characterized by enhanced IL-12p70 production (Fig 1C) and tumor-specific CTL responses (Fig 1D), compared to immature (i)DCs and non-polarized standard (s)DCs matured by the IL-1β/TNF-α/IL-6/PGE_2 cocktail commonly used in DC vaccine trials[9]. Alternatively, IL-2 selectively primes NK cells for potent cytotoxic activity (NK_k; Fig 1A) without substantially enhancing their IFNγ production (Fig 1B) or DC-activating capacity, suggesting differential regulation (and thus, possible therapeutic separation) of these lytic and non-lytic NK cell activities.

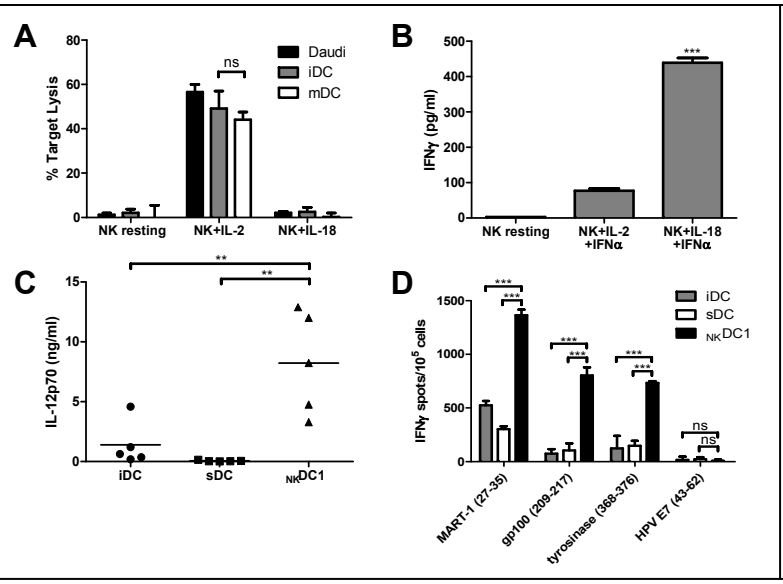


Fig 1. NK_k cells efficiently kill tumor cells and both immature and mature DCs, while NK_h cells selectively produce high levels of IFNγ and generate type-1-polarized DCs (DC1s) that are potent inducers of tumor-specific CTLs. (A) Cytotoxicity against Daudi tumor cells, iDCs, or LPS-matured (m)DCs by NK cells stimulated with IL-2 (250 IU/ml) or IL-18 (100 ng/ml) for 48h. (B) IFNγ in 24h culture supernatants from NK cells stimulated with IL-2 (250 IU/ml) or IL-18 (100 ng/ml), and IFNα (1000 IU/ml). (C) IL-12p70 in 24h culture supernatants from CD40L-stimulated iDCs, IL-1β/TNF-α/IL-6/PGE_2-matured sDCs, or NK_h-activated $_{NK}$DC1s; aggregate data from 5 stage III-IV melanoma patients. (D) Autologous $CD8^+$ T cells were sensitized with differentially-activated DCs loaded with HLA-A*0201-restricted melanoma-associated peptides and analyzed by IFNγ ELISPOT against melanoma-associated peptide-loaded T2 cells. ***p<0.001, **p<0.01, *ns* p>0.05.

Distinct immune synapses have recently been reported in differentially-mediating lytic and non-lytic NK cell functions during their reciprocal interaction with macrophages[25], and a unique DC-NK 'regulatory' synapse has been recently described governing the IL-15Rα-mediated survival of NK cells that is qualitatively different from the classical NK synapses formed in association with MHC class I^{low} targets[28]. This suggests that NK cells may form different types of immune synapses with DCs and tumor cells depending on the functional intent of their interactions. If known, this may provide critical targets for limiting the undesirable DC-killing activity of NK cells while maintaining or enhancing the desirable tumor killing and DC-activating capacity of NK cells.

I.B.5. Suppressive MDSCs in human ovarian cancer (OvCa) and the key role of prostaglandin E_2 (PGE_2). MDSCs are a heterogeneous population of myeloid cells present in most cancer patients and animal models of cancer. Characterized primarily by their potent ability to suppress T cell activity and their poorly-differentiated, immature state, they are recognized as essential contributors to tumor environment immune-suppression and are considered a major impediment to effective anti-cancer immunotherapy, including NK- and DC-based therapy[10,29]. This highlights the critical need to study the interaction of these cells with MDSCs. Human OvCa is known, in particular, to be greatly exacerbated by the highly-immune-suppressive nature of the peritoneal tumor environment, and our preliminary data indicate elevated numbers of MDSCs in OvCa ascites, identified by a $CD11b^+CD33^+CD34^+CD14^+HLA\text{-}DR^{low}$ phenotype with low/absent expression of CD80, CD83, and CD86 (Fig 2A), features associated with the monocytic subset of human MDSCs[30]. These cells highly express many MDSC-associated suppressive factors (Fig 2A-B) and are potent suppressors of $CD8^+$ T cells (Fig 2C). Thus, these cells provide an attractive model to study MDSC interactions relevant to the human tumor environment and to cellular immunotherapy. Consistent with prior work demonstrating the key influence of tumor-associated inflammation in MDSC induction[31], our preliminary data also suggest a critical role for the inflammatory lipid PGE_2 in the induction and maintenance of these MDSCs in the peritoneal cavity (Fig 2B-C).

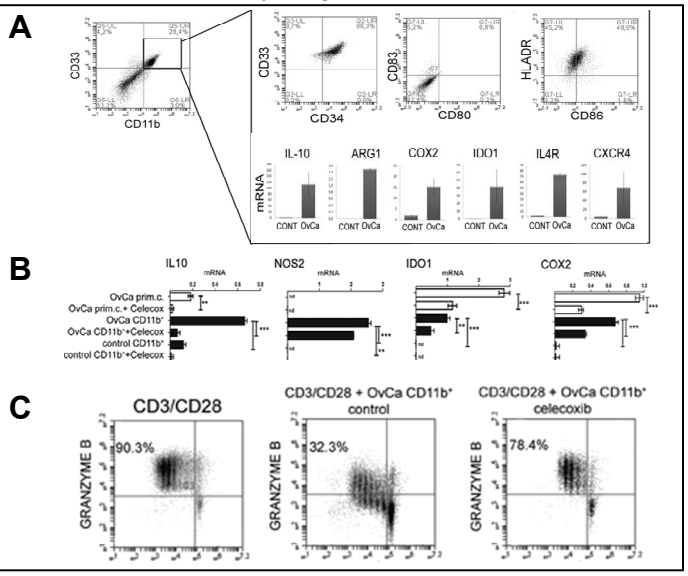


Fig 2. Enriched population of MDSCs in human ovarian cancer (OvCa) ascites and the dependence of their suppressive functions on COX2 activity. (A) $CD11b^+$ cells isolated from OvCa ascites were analyzed for surface phenotype and expression of MDSC-associated suppressive factors, compared to control $CD11b^+$ cells isolated from healthy donor peripheral blood. Note uniform MDSC phenotype and high suppressive molecule expression by ascites-derived $CD11b^+$ cells. (B) Effect of celecoxib (COX2 inhibitor; treated at 20 μM for 16h) treatment on the expression of MDSC-associated suppressive factors by bulk primary cells from OvCa ascites, $CD11b^+$ cells isolated from OvCa ascites, or control $CD11b^+$ cells isolated from healthy donor peripheral blood. (C) Effect of celecoxib treatment on the suppression of CD3/CD28 bead-stimulated $CD8^+$ T cell proliferation and granzyme B expression by co-cultured $CD11b^+$ cells isolated from OvCa ascites or control healthy donor peripheral blood. Note abrogation of suppressive factor expression and CTL-suppressing activity by COX2 blockade with celecoxib. ***p<0.001, **p<0.01

I.B.6. MDSC interactions with differentially-activated NK cells. Current understanding of MDSC interactions with NK cells remains limited, especially in humans. Prior studies, largely in mouse models, have focused almost exclusively on the suppressive effect of MDSCs on NK cells[32-35], with no published studies describing the reciprocal effect of activated NK cells on MDSC function. Elucidating this effect will be vital to understanding the complex reciprocal biology of key immune-modulatory cells in the tumor environment as well as to understanding the potential influence of NK cell-incorporating therapies on tumor-associated immune-suppression. Our preliminary data suggests that, in analogy to NK cell interaction with DCs, IL-2-activated 'killer' NK cells can exhibit potent cytotoxicity against MDSCs (Fig 3A). However, in contrast to their desirable immune-stimulatory effect on DCs, IL-18-activated 'helper' NK cells may instead undesirably enhance MDSC-mediated suppression through the potent induction of MDSC suppressive factors, including indoleamine 2,3-dioxygenase (IDO) and the essential PGE_2-producing enzyme, cyclooxygenase-2 (COX2) (Fig 3B). Defining the underlying mechanisms, particularly the role of the COX2-PGE_2 axis, will be critical to optimizing NK cell-incorporating therapeutic strategies, helping to identify means to selectively suppress NK-dependent MDSC activation while preserving NK-mediated DC activation to promote productive NK-DC therapeutic feedback.

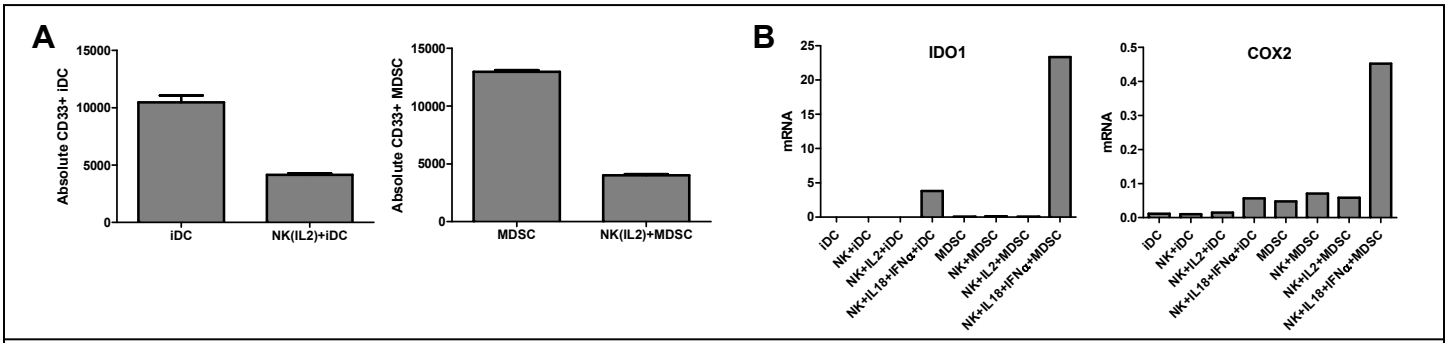


Fig 3. NK_k cells efficiently kill both immature DCs and MDSCs, while NK_h cells enhance the expression of MDSC-associated suppressive factors. (A) Cytotoxicity against iDCs (left) or MDSCs (right) by NK cells stimulated with IL-2 (250 IU/ml) for 48h. (B) Gene expression (relative to HPRT1) of MDSCs co-cultured for 24h with NK cells treated with IL-2 (250 IU/ml) or IL-18 (100 ng/ml) and IFNα (1000 IU/ml). Note enhancement of IDO1 and COX2 expression in MDSCs co-cultured with IFNγ-producing NK cells treated with IL-18/IFNα.

Overall, the proposed studies will mechanistically address important unresolved biology in the interaction between NK cells, tumor cells, and two cell types (DCs and MDSCs) highly relevant to the immunology of human cancer, and directly contribute to the design of more effective cell-based cancer therapies.

I.C. Approach

The overall strategy of the proposed Specific Aims (SAs) focuses on NK cell-mediated regulation of the survival and function of two cell populations (DCs and MDSCs) with key opposing roles in the immune system, with specific implication for the optimization of NK cell- and DC-based immunotherapy. In SA1, I will address the hypothesis that the desirable NK cell abilities to kill tumor cells and activate/polarize DCs can be mechanistically separated from the undesirable NK cell ability to kill DCs (particularly immune-stimulatory mature DCs), providing prospective therapeutic targets for the selective modulation of these different activities. In SA2, I will address the complementary finding that, in contrast to their desirable effect on DC activation, 'helper'-activated NK cells may in fact contribute to an undesirable enhancement of MDSC-associated immune-suppression. I will further test the hypothesis that this undesirable NK cell effect on MDSC function may be uncoupled from the desirable NK cell-mediated enhancement of DC activation, by modulation of the COX2-PGE_2 axis selectively activated in MDSCs. Jointly, the questions addressed in SA1 and SA2 will allow me to elucidate unique mechanistic means by which to selectively enhance the desirable immune-stimulatory functions of activated NK cells (tumor-killing, MDSC-killing, and DC activation) while minimizing the undesirable immune-suppressive NK cell functions (DC-killing and MDSC activation). This approach will provide extensive training in contemporary immunology research techniques applicable to a planned future career in translational cancer research (including biologic imaging, cellular function assays, genetic and protein analysis, and work with primary human cancer material) as well as ample opportunity for diverse data collection and analysis, forming the basis for both research and professional skill development.

I.C.1. Specific Aim 1: Define the mechanisms involved in 'killer' NK cell and 'helper' NK cell function during NK cell interaction with DCs and tumor cells.

Hypothesis: Specific independently-regulated factors may be involved in the selective ability of NK cells under different patterns of activation to activate DCs as well as to kill tumor cells and both immature and mature DCs.

Rationale and Significance: Our prior work[16,27] and new preliminary data (Fig 1) demonstrate that different patterns of NK cell activation can result in divergent NK-DC interactions, including both DC activation and DC killing. However, the mechanisms underlying these differential interactions, particularly the critical surface molecules and apoptosis-inducing mechanisms, are still unclear. Identifying factors selectively controlling the desirable (tumor-killing and DC-activating) and undesirable (DC-killing) functions of NK cells will help optimize NK- and DC-based immunotherapies by providing potential targets for selective modulation of these distinct NK cell functions. Differential NK cell priming with IL-2 or IL-18 into distinct killer or helper phenotypes[27] (and Fig 1) offers an attractive model to directly assess these mechanisms.

I.C.1.a. Identify and compare the effector mechanisms involved in the NK cell killing of DCs and tumor cells. Resting NK cells isolated from healthy donors will be cultured for 48h with IL-2 (250 IU/ml) or IL-18 (100 ng/ml), with and without IFNα (1000 IU/ml) as a secondary signal[16], to produce the respective cytolytic and helper NK phenotypes[27]. Untreated NK cells will serve as controls. Autologous iDCs will be generated from $CD14^+$ monocytes cultured for 6d in GM-CSF (1000 IU/ml) and IL-4 (1000 IU/ml), and further treated with LPS (250 ng/ml) for 48h to generate mature (m)DCs[16]. Resting or differentially-activated NK cells will be co-cultured with iDCs, mDCs, or tumor cells (Daudi, SLM2 melanoma, HT29 colorectoral, and/or SKOV3 ovarian tumor lines, relevant to our clinical trials) at 1:5, 1:1, and 5:1 ratios. NK cytotoxicity will be assessed by 4h ^{51}Cr-release assays and 7-AAD staining of Cell Tracker Orange (CTO)-labeled targets[36], with K562 cell killing by IL-2-treated NK cells as a positive control. The DC-activating capacity of NK cells will be assessed by flow cytometry for DC expression of maturation-associated CD80, CD86, CD83, CD40, HLA-DR, and CCR7, as well as by ELISA for DC IL-12p70 production, the key type-1-polarizing factor critical for anti-tumor immunity, after stimulation with CD40L-transfected J558 cells (a well-validated surrogate for $CD4^+$ T cell co-interaction[37]). High IL-12-producing αDC1s (IL-1β/TNFα/IFNα/IFNγ/poly-I:C-treated DCs[38]) will act as a positive control.

Within this system, I will compare the cytolytic mechanisms used by differentially-activated NK cells against tumor, iDC, and mDC targets, focusing on perforin, granzyme, FasL, and TRAIL-dependent pathways (see I.B.3). To assess perforin and granzyme B (GrB) involvement, I will use CMA and/or EGTA treatment to inhibit active perforin[39], and use membrane-permeable peptide inhibitors of GrB (z-AAD-CMK, IETD-CHO, and/or z-IETD-fmk)[26,40]. Neutralizing FasL mAbs will be used to assess FasL-dependent killing[39], and TRAIL-dependent death will be assessed through relevant blocking mAbs to TRAIL or the TRAILR1 and TRAILR2 receptors. Guided by these studies and the lytic pathways implicated, I will also examine the possible active protection of DCs by NK cell help, specifically the induction of PI-9, c-FLIP, c-IAP2, Bcl-2, and Bcl-xL, known to promote DC survival[26,41-43], using quantitative mRNA and immunoblot analysis.

I.C.1.b. Identify and compare the NK cell surface factors involved in NK cell killing of DCs and tumor cells and NK cell activation of DCs. Using confocal microscopy, I will characterize and compare the synapses formed between IL-2- and IL-18-activated NK cells and tumor cells, iDCs, and mDCs at fixed time points (0.5h and 2h, optimal in prior studies[44]). I will focus on intracellular mobilization of TNFα and IFNγ (representative immune-stimulatory 'helper' molecules) and key lytic molecules determined by I.C.1.a. Concurrently, I will assess the synaptic composition of a limited set of surface molecules implicated in NK lytic and non-lytic activity during tumor and DC interaction, specifically NKG2D, NKp46, NKp30, DNAM-1, 2B4, KIR2DL4, and LFA-1[21-23,45-47]. For individual receptor assessment, I will expose resting or IL-2 or IL-18-activated NK cells to biotinylated agonist mAbs to these receptors conjugated to streptavidin-coated polystyrene beads[48], followed by confocal analysis of TNFα, IFNγ, and lytic factor mobilization to the NK/bead interface. In complement, imaging of differentially-activated NK cells with tumor and DC targets will be similarly performed with blocking mAbs to candidate receptors, with functional confirmation by the cytolytic and DC-activating readouts described above. The success of the indicated imaging studies will be supported by focused technical courses in biologic imaging techniques offered through the Center for Biologic Imaging.

Anticipated Results: This analysis will identify the predominant and potentially distinct pathways underlying NK cell cytotoxicity against DCs and tumor cells. This analysis will also define the critical and perhaps distinct NK-derived surface factors selectively underlying the DC-activating functions and DC-killing functions of NK cells. The results of this aim would thus provide unique scientific insights into target-specific NK cell cytotoxicity as well as uncover potential mechanistic means to uncouple the desirable tumor-killing and DC-activating functions of NK cells from their undesirable DC-killing functions. This would contribute to the improvement of cell-based cancer immunotherapy by informing specific approaches to therapeutic NK cell activation or the selective inhibition of undesirable NK cell functional pathways using pharmacologic or antibody-based inhibitors. These results will also help to mechanistically prioritize the complementary NK-MDSC analyses of SA2 and contrast the opposing NK cell interactions with DCs and MDSCs for potential therapeutic targeting.

Potential Pitfalls and Alternative Strategies: The initial time points selected above for confocal analysis may not optimally reveal differences in the interactions between differentially-activated NK cells and tumor and DC targets, as lytic and non-lytic interactions have been described at even shorter or longer intervals[25,49]. In this case, I will extend our range of time points (10min – 4h), guided by additional live cell imaging assessing certain informative parameters, such as membrane blebbling. Furthermore, if my analysis does not reveal the critical signals regulating specific NK cell activities, I will analyze additional candidates less-strongly associated with the selective governing of lytic and non-lytic NK functions, including CD2 and LLT1[46,50].

I.C.2. Specific Aim 2: Define the outcomes of activated NK cell interaction with MDSCs in the setting of human ovarian cancer (OvCa) and characterize the mechanisms governing these interactions.

Hypothesis: Differentially-activated NK cells may differentially-modulate MDSC suppressive activity, influencing the outcome of NK cell-mediated DC activation. Furthermore, disruption of the COX2-PGE_2 axis may prevent undesirable NK-mediated MDSC activation while preserving desirable NK-mediated DC activation.

Rationale and Significance: Our preliminary data suggests the high prevalence of MDSCs in the OvCa peritoneal environment (Fig 2), and given their key influence on tumor immunity[29], elucidating their full interaction with immune effectors is vital to understanding tumor environment biology and enhancing cancer immunotherapies. Current understanding of MDSC interactions with NK cells is limited, with no studies addressing the effect of activated NK cells on MDSC functions. Guided by our preliminary data indicating the potential of differentially-activated NK cells to either kill or hyper-activate MDSCs (Fig 3), I will systematically assess the impact of NK cells under different patterns of activation on MDSC suppressive functions, and the effect of NK-mediated MDSC activation on DC immune-stimulatory capacity. Further guided by our preliminary data suggesting the essential role of sustained PGE_2 in maintaining MDSC functions (Fig 2), I will also assess whether inhibition of the COX2-PGE_2 axis may reverse the undesirable NK-mediated enhancement of MDSC activity, while preserving the desirable NK-mediated activation of DC immune-stimulatory function. Our access to MDSC-rich OvCa ascites provides an accessible, clinically-relevant model to study these interactions.

I.C.2.a. Characterize the effect of differentially-activated 'killer' and 'helper' NK cells on the suppressive activity of OvCa-associated MDSCs. Analogous to I.C.1, I will differentially activate, or leave untreated, NK cells with IL-2 or IL-18, with and without IFNα as a secondary signal, and co-culture them with magnetically-isolated $CD11b^+$ MDSCs from OvCa ascites (Fig 2) or control $CD11b^+$ cells from healthy donors. I will assess cytotoxicity against labeled MDSCs (as in I.C.1.a) and the expression of MDSC-associated suppressive factors (including IDO, ARG1, iNOS, IL-10, TGF-β) by quantitative mRNA, immunoblot, ELISA, and intracellular staining, where appropriate. To assess whether exposure to differentially-activated NK cells modulates the active ability of MDSCs to suppress T cells, I will add CSFE-labeled CD3/CD28 bead-stimulated $CD8^+$ T cells to these NK-MDSC co-cultures, or to control NK cell co-cultures with $CD11b^+$ cells from healthy donors, and monitor T cell proliferation (by CSFE dilution) and cytolytic potential (by intracellular staining of GrB). To define the critical mechanisms underlying the induction and performance of this MDSC suppression, I will perform blocking studies, focusing on the role of IFNγ and TNFα (using blocking mAbs or soluble IFNγR1 and TNFR1) in MDSC activation, and IDO, ARG1, iNOS, IL-10, and TGF-β in MDSC-mediated T cell suppression (using nor-NOHA, L-NMMA, and L-1MT inhibitors and IL-10 and TGF-β blocking mAbs, respectively).

Finally, most relevant to NK and DC-based cancer immunotherapy, and directly complementary to SA1, I will examine the net effect of differentially-activated NK cells and MDSCs on modulating DC secretion of IL-12p70 (analogous to I.C.1.a) during triple cultures of NK cells, MDSCs, and DCs. Within this model, I will also assess the DC ability to induce Th1 and CTL responses during *in vitro*-stimulation of naïve $CD4^+$ and $CD8^+$ T cells, facilitated by the commonly-used Staphylococcal enterotoxin B (SEB) superantigen model[26]. T cell readouts will include ELISA analysis of IFNγ vs. IL-5 secretion, flow cytometric analysis of viability/proliferation and intracellular perforin and GrB, and cytotoxicity against SEB-pulsed JY cell targets.

I.C.2.b. Determine the effect of COX2-PGE_2 blockade on NK cell-mediated hyper-activation of MDSCs. Guided by our preliminary data suggesting the essential requirement for sustained PGE_2 signaling in the maintenance of MDSC suppressive function (Fig 2), I will use the culture design and functional suppression readouts outlined in I.C.2.a to examine the ability of COX2-PGE_2 blockade to prevent NK-mediated hyper-action of MDSC suppressive function. Specifically, using celecoxib-mediated COX2 inhibition and/or AH23848 and AH6809 antagonists of the EP2/4 PGE_2 receptors, I will assess the impact of COX2-PGE_2 blockade on activated NK cell-induction of MDSC suppressive molecules and MDSC-mediated CTL suppression. I will also investigate the impact of COX2-PGE_2 blockade on the ability of DCs to secrete IL-12p70 and generate Th1 and CTL responses in the NK, MDSC, and DC triple-culture model described above.

Anticipated Results: I anticipate that IL-18-activated NK cells can enhance MDSC suppressive activity, and

expect to define the predominant mechanisms of its induction and performance. This analysis is likely to reveal at least some candidate suppressive pathways induced by NK cell-mediated MDSC activation, such as IDO induction, which may be amenable to selective therapeutic modulation without compromising the desirable NK cell ability to enhance immune-stimulatory DC activation. Based on our preliminary data suggesting a critical requirement for sustained COX2-PGE_2 activity in maintaining the suppressive functions of OvCa-derived MDSCs (Fig 2), I also anticipate that inhibition of the COX2-PGE_2 axis will reverse the undesirable augmentation of MDSC suppressive activity by IL-18-activated NK cells, while preserving and/or enhancing their desirable DC-activating functions. These studies will thus contribute significantly to the optimization of productive NK-DC immunotherapeutic feedback in the setting of tumor-associated immune-suppression.

Potential Pitfalls and Alternative Strategies: Although our preliminary data (Fig 2) indicate the high prevalence of MDSCs within the $CD11b^+$ fraction of OvCa ascites, in a limited set of experiments, I may further compare bulk $CD11b^+$ cells against flow-sorted $CD11b^+CD33^+$ $CD124^+CD80^-CD83^-$DC-$SIGN^-$ ascites cells, proposed as human MDSC markers[30], to assess potential contaminating cell effects. The use of SEB as a polyclonal model of DC-driven T cell activation will greatly facilitate the practical analysis of the effect of activated NK cells and MDSCs on DC immune-stimulatory capacity, but may be affected by the peptide-non-specific nature of the DC-T cell interaction. Thus, in a limited set of experiments, I may further compare DCs derived from HLA-A^*0201^+ donors loaded with OvCa-relevant HLA-A*0201-restricted HER2/neu and survivin peptides (in analogy to melanoma-relevant peptides used in our prior studies, see Fig 1) to activate antigen-experienced T cells in a peptide-specific manner. Furthermore, the described interactions may be influenced by autologous/allogeneic settings, analysis of which may be hindered by the availability of clinical material. If so, I will use healthy donor-derived NK cell, DCs, and/or T cells and autologous *in vitro*-derived MDSCs to model these interactions, generated from monocytes treated during GM-CSF/IL-4 differentiation with OvCa ascites, media conditioned with OvCa cells, or PGE_2, all displaying a similar phenotype as primary MDSCs (data not shown).

The joint results of SA1 and SA2 will ultimately inform the development of a single cohesive therapeutic strategy combining a 'preferred' mode of NK cell activation with rationally-selected pharmacologic or antibody-based functional modifiers to boost the desirable anti-cancer activities of NK cells (tumor-killing, MDSC-killing, and DC activation) while limiting their undesirable immune-suppressive activities (DC-killing and MDSC activation). The resulting paradigms will form the basis for further development within the UPCI to modify ongoing cell-based immunotherapeutic strategies for the treatment of cancer.

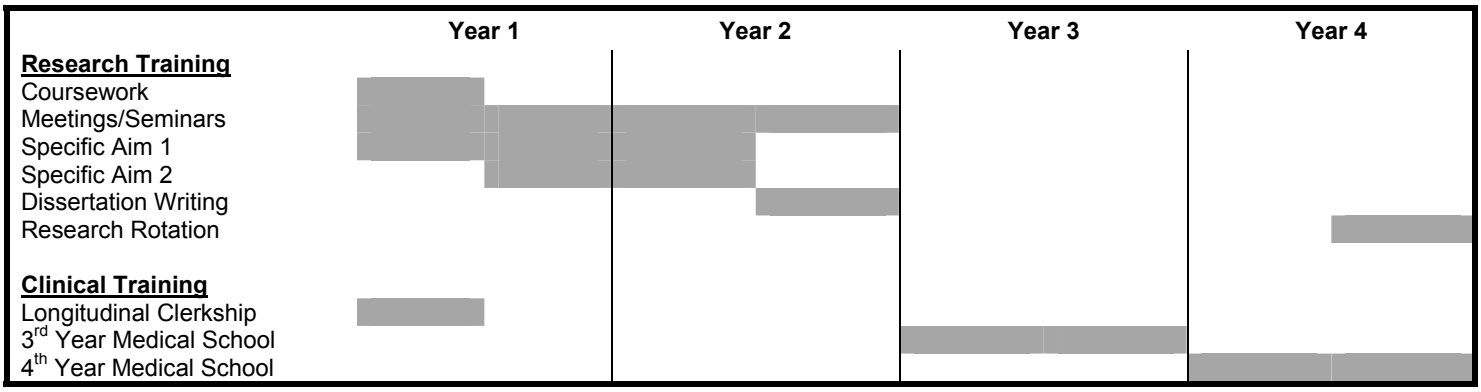
I.D. Statistical considerations

All quantitative data, including mean fluorescence intensities from flow cytometry, optical absorbance from ELISA, amplification threshold values from RT-PCR analysis, densitometric units from immunoblot analysis, gamma emission counts from ^{51}Cr-release assays, and spot counts from ELISPOT assays will be analyzed statistically by one- or two-way ANOVA as appropriate for the analysis of one or two variables, respectively. Significance will be judged at an α of 0.05.

I.E. Biohazard considerations

Risks associated with the use of chemical, radioactive, and biological hazards, including dimethyl sulfoxide, sodium azide, ^{51}Cr radioactive isotopes, primary human biologic material, and human cell lines will be minimized by universal handling precautions, personal protective equipment, dedicated workspaces (for instance, for radiation work), and proper storage, inventory, and disposal of biohazard material. See "Select Agent Research" for an additional discussion of the use of SEB, a DHHS Select Toxin.

I.F. Tentative sequence



I.G. Human Subjects Research

I.G.1. Protection of Human Subjects

I.G.1.a. Risks to Human Subjects

I.G.1.a.1. Human Subjects Involvement and Characteristics

A major portion of the proposed Research Strategy will be performed using human buffy coats (a waste product of the preparation of coagulation factors) derived from voluntarily-donated peripheral blood from healthy individuals, purchased from the Central Blood Bank of Pittsburgh and provided without any personal identifiers. Specific Aim 2 of the proposed research will additionally employ tumor and ascites samples, as well as peripheral blood, harvested from patients undergoing standard-of-care surgeries for the treatment of ovarian cancer. This material will be obtained from 40 patients, using the IRB-approved UPCI protocol #02-077 (IRB021068, approval date 3/18/11). The number of patients is based on prior participation in this banking protocol projected over the duration of the proposed fellowship period. Potential subjects will be identified by the examining physician, and eligibility criteria will include patients without known immunodeficiency conditions, such as HIV infection, due to the nature of the proposed research studies (investigating immune cell interactions relevant to ovarian cancer). Informed consent will be obtained on all subjects by the protocol principal investigator or co-investigators prior to all screening procedures, and no patient will be entered into the protocol without having a signed written consent form. Due to the character of the disease, the proposed protocol will involve women only. The racial and ethnic characteristics of the proposed subject population will reflect the demographics of Pittsburgh and the surrounding area and/or the patient population of the UPMC Health System. Every attempt will be made to recruit subjects in respective proportions to these demographics, and every attempt will be made to encourage participation of minority subjects. No patients will be excluded based on race, ethnicity, or socioeconomic status.

I.G.1.a.2. Sources of Materials

Materials used in the proposed studies include peripheral blood (obtained by venipuncture) and ovarian cancer tumor and ascites material (obtained as a product of surgeries provided as standard-of-care for the treatment of ovarian cancer). No data will be collected from subjects specifically for the proposed research project. No additional data will be collected from subjects beyond data collected as part of the clinical care of the patient. Access to this patient information will be granted only to authorized personnel participating in the clinical care of the patient, in full accordance with appropriate HIPAA guidelines. Access to this patient information will be kept in a secure location inaccessible to unauthorized individuals.

I.G.1.a.3. Potential Risks

Tumor and ascites material will be harvested within surgeries provided as standard-of-care for the treatment of ovarian cancer, with no additional risk incurred beyond those associated with surgical treatment. Venipuncture to obtain peripheral blood is a minimal risk procedure, associated rarely with infection or fainting (<1%) or bruising, bleeding, and/or soreness (<10%).

I.G.1.b. Adequacy of Protection Against Risks

I.G.1.b.1. Recruitment and Informed Consent

Potential subjects will be identified by the examining physician (according to the criteria described above), and written informed consent will be obtained on all subjects by the protocol principal investigator or co-investigators prior to all screening procedures. Risks (described above) and benefits (described below) will be described to subjects during the consent process.

I.G.1.b.2. Protections Against Risk

Venipuncture will be performed only by fully-trained phlebotomists to minimize risks of the procedure. Every effort will be made to preserve confidentiality of the records of the patients enrolled in the protocol. Access to patient information will be kept in a secure place inaccessible to unauthorized individuals. Access to this information will be granted only to authorized personnel participating in the clinical care of the patient, in full accordance with appropriate HIPAA guidelines.

I.G.1.c. Potential Benefits of the Proposed Research to Human Subjects and Others

There are no direct benefits to donors of blood or tissue materials. Knowledge gained from the proposed studies using these materials may help improve future cancer therapies (see below). Although no direct benefit exists, the minimal risks (described above) of participation in the protocol are reasonable in relation.

I.G.1.d. Importance of the Knowledge to be Gained

Knowledge gained from the proposed studies using these materials may help improve future cancer treatment by elucidating critical immune-stimulatory and immune-suppressive processes relevant to human ovarian cancer. Knowledge from these studies may also potentially provide therapeutic targets for enhancing productive anti-cancer immune cell interactions, helping to improve future cancer immunotherapies. The minimal risks (described above) associated with participation in the protocol are reasonable in relation to the importance of this knowledge for improving future cancer therapy.

I.G.2. Inclusion of Women and Minorities

Due to the character of the disease (ovarian cancer), the proposed protocol will involve women only. The racial and ethnic characteristics of the proposed subject population will reflect the demographics of Pittsburgh and the surrounding area and/or the patient population of the UPMC Health System. Every attempt will be made to recruit subjects in respective proportions to these demographics. No patients will be excluded based on race, ethnicity, or socioeconomic status. Targeted/planned enrollment by race is provided in the Targeted/Planned Enrollment Table.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title: Stimulatory and Suppressive NK Cell, DC, and MDSC Interactions in Human Cancer

Total Planned Enrollment: 40

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	3	0	3
Not Hispanic or Latino	37	0	37
Ethnic Category: Total of All Subjects *	40	0	40
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	1	0	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	5	0	5
White	34	0	34
Racial Categories: Total of All Subjects *	40	0	40

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

I.G.3. Inclusion of Children

Because the incidence of ovarian cancer under the age of 20 is extremely rare (SEER incidence around 0.5 per 100,000), no children will be enrolled in the protocol.

I.H. Vertebrate Animals

Not applicable.

I.I. Select Agent Research

The proposed studies employ the use of Staphylococcal enterotoxin B, which is considered a DHHS Select Toxin under 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73. However, the amount under control of the applicant and the sponsor's lab will not exceed, at any time, 5 mg, and is thus not regulated by the DHHS. Nevertheless, appropriate precautions will be used when storing, handling, and disposing of this material, including monitored storage in a secure location accessible only by authorized users, appropriate use of protective equipment (ventilated hood with eye protection, lab coat, and latex gloves), and proper disposal in appropriate biohazard waste containers. These procedures are consistent with the general safety precautions which will be used during the proposed studies to protect from risks associated with the use of laboratory chemical, radioactive, and biological hazards, including work with dimethyl sulfoxide, sodium azide, ^{51}Cr radioactive isotopes, primary human biologic material, and human cell lines. These procedures include universal handling precautions, performance of studies in designated locations with designated equipment (for instance, in chemical or biologic hoods or designated radiation workbenches), use of personal protective equipment (as described above), and proper storage, inventory, and disposal of biohazard material.

I.J. Resource Sharing Plan

Data Sharing Plan

All data generated from work performed for this proposal will be freely available. The data will be primarily distributed through abstract presentations at institutional, regional, national, and international meetings and through manuscript publication in peer-reviewed scientific journals.

Sharing Model Organisms

The development of a model organism is not anticipated.

I.K. Respective Contributions

The specific aims and the entirety of the research training plan were developed and written by the applicant, Jeffrey Wong, with assistance and editorial input from the sponsor, Dr. Pawel Kalinski. Dr. Kalinski provided valuable feedback in the development and fine-tuning of the proposal through discussions of pertinent preliminary data, the current state of cell-based cancer immunotherapy, contemporary scientific issues related to the proposal, and resources available for supporting any proposed studies. In reviewing this proposal, Dr. Kalinski also suggested content and formatting changes to improve the overall focus and clarity. The proposed fellowship work will be a closely-mentored research experience encouraging independent initiative in the design and completion of the studies, but with frequent sponsor input and advice through weekly formal meetings and more frequent informal meetings, as appropriate (please see “Training Plan” section II.C.1 for additional detail).

I.L. Selection of Sponsor and Institution

My ultimate goal is to become an independent physician-scientist devoted to integrating the full spectrum of innovative patient care, clinical trial involvement, and translation-driven bench-based research for the treatment of cancer. In the pursuit of this goal, I am seeking a sponsor that has a career-long experience with lab-based tumor immunology research as well as direct and leading involvement in the translation of these findings to therapeutic clinical studies. Furthermore, given the accelerated nature of dual-degree training, I am seeking a sponsor that already has considerable experience specifically in training researchers with a heavily-translational research focus and a clear goal of integrating clinical practice into a future career. In support of this sponsor experience, I am also seeking a carefully-structured dual-degree training program designed specifically to develop the unique skillset necessary for research/clinical integration, set within an institutional atmosphere that is supportive (in both resources and expertise) of an individualized training program in cancer research and clinical cancer care.

Dr. Pawel Kalinski, MD, PhD (Professor of Surgery, Immunology, and Infectious Disease & Microbiology; Director of Research, Division of Surgical Oncology) is an ideal sponsor for this fellowship training. He is a recognized research leader in human tumor immunology and cell-based cancer immunotherapy, particularly as it relates to dendritic cells and dendritic cell vaccines. His funding history and past publications demonstrate a strong record of research success and considerable experience in studying effector cell (including natural killer cell) interactions with dendritic cells and other myeloid cells (which is the conceptual focus of this proposal), including past publications in the *Journal of Experimental Medicine* directly relevant to this proposal's aims. Supporting his role as an ideal mentor for translational cancer research, he has an extensive network of clinical collaborators, including as Director of Research for the Division of Surgical Oncology and in close associations with clinical programs in the University of Pittsburgh Cancer Institute and the Magee-Womens Hospital. These connections provide a unique opportunity to strengthen my physician-scientist mentorship as well as access patient-derived human cancer material critical to the success of my translational research training. He is also extensively involved in clinical trials directly transitioning bench-based advances in the polarization and chemokine-axis modulation of dendritic cells for cancer therapy (funded by P01, R21, and foundational mechanisms). This involvement further provides the opportunity to directly experience the development, implementation, and interpretation of clinical trials and clinical trial questions. The success of his past trainees, many of which are current academic faculty in integrated research and clinical roles, demonstrates mentorship experience ideally suited for my particular career trajectory.

The mentorship of Dr. Kalinski is complemented by a Medical Scientist Training Program (MSTP), led by Dr. Clayton Wiley, MD, PhD (Director of the MSTP and Associate Dean, School of Medicine), that is routinely at the forefront of dual-degree training innovations, committed to the integration of clinical and research training and the active development of the professional and ethical skills essential for success as a future independent physician-scientist. Longitudinal enrichment activities (see "Training Plan" section II.C.1) include "Research Basis of Medical Knowledge" courses during the first two years of medical school providing training in the critical interpretation of seminal biomedical research papers; multiple research rotations to facilitate the development of research skills and identification of thesis labs; professional development courses devoted to scientific communication, networking, and grant-writing and ethical reasoning; structured longitudinal clinical clerkships during graduate school to foster clinical skills and research/clinical integration; monthly MSTP seminars/workshops providing structured access to physician-scientist role models and peer-driven professional and ethics development; and yearly MSTP retreats offering a dedicated MD-PhD forum for scientific and mentoring interactions.

The strength of the MSTP is further enhanced by its association with the University of Pittsburgh Medical Center (UPMC), the University of Pittsburgh School of Medicine (which also contains the Immunology Graduate Program), and the University of Pittsburgh Cancer Institute (UPCI), which are collectively home to one of the most comprehensive medical research and clinical infrastructures in the country (see "Environment" section II.C.2). Through the leadership of Dr. Arthur Levine, MD (Dean, School of Medicine) and Dr. Nancy Davidson, MD (Director, UPCI), I will benefit from a strong institutional emphasis on translational biomedical research especially in the cancer field, emphasis on the specific career development of academic physician-scientists, and a rich UPMC medical and patient environment supporting top-notch clinical training and opportunities for translational research.

For these reasons, I believe I have selected an exceptionally-qualified sponsor within a uniquely-tailored, nationally-recognized dual-degree training environment ideal for my development as a physician-scientist in translational cancer research.

I.M. Responsible Conduct of Research

Extensive training in the responsible conduct of research is structured within the required curriculum and enrichment activities of the Medical Scientist Training Program (MSTP). This training is provided longitudinally throughout the medical and graduate school phases.

As part of the first year medical school curriculum (Fall 2007), I completed a course entitled “Ethics, Law, and Professionalism” designed to explore the legal, ethical, and professional issues that define the practice of medicine and the performance and application of biomedical research. The course consisted of 15 two-hour sessions providing instruction in a variety of formats, including lectures, large-group panel discussions, and small-group faculty-facilitated discussions, led by diverse faculty from the University of Pittsburgh Center for Bioethics and Health Law and the University of Pittsburgh School of Medicine. The course provided an introduction to the legal system and principles of ethical analysis most relevant to clinical practice and biomedical research. Within this framework, a series of key issues facing physicians and physician-researchers were discussed, including informed consent, confidentiality, treatment of vulnerable populations, ethical implications of emerging medical technologies, and physician and research interactions with the pharmaceutical industry.

During the first year of graduate school (May 2010), I also completed an MSTP-designed course entitled “Ethics for Medical Scientists”, organized and directed by Rita Pinkus, PhD (Associate Director of the Center for Bioethics and Health Law) and Richard Steinman, MD, PhD (Co-Director of the MSTP) in collaboration with the student-run MSTP Ethics Committee. The course consisted of 4 two-hour sessions driven by faculty-facilitated student discussion, intended to introduce general ethical concepts, analytical methods in the evaluation of ethical dilemmas, and the legal, philosophical, and personal components of ethical decision-making. These concepts were then applied to the evaluation of contemporary issues in biomedical research and clinical practice, including animal experimentation, considerations in human subject research, conflicts of interest, and the interface of research and clinical medicine with biotechnology, among other topics.

As part of my graduate research experience, I also completed 4 internet-based training modules most relevant to my research work, covering principles of research integrity, human subject research in biomedical science, conflicts of interest, and HIPAA privacy as it relates to research. The School of Medicine curriculum additionally requires the completion of internet-based training modules covering the legal guidelines governing the interaction between the pharmaceutical industry and physicians and physician-researchers; ethical interactions between students, faculty, and research subjects; and the association of academic research with private sector development.

To supplement these experiences, the MSTP further devotes two sessions per year of its monthly MSTP seminar/workshop series to the discussion of ethical issues in clinical medicine and research, facilitated by prominent bioethics faculty at the University of Pittsburgh. Past sessions have included such topics as the responsible reporting of research results for publication, conference presentation, and grant submission; historical perspectives on human subject research; HIPAA and IACUC guidelines; collaboration in research science; and mentor and mentee relationships. By the time of this award, I will have participated in 8 of these sessions, and will participate in an additional 8 sessions during the fellowship period.

Goals for Fellowship Training and Career

My ultimate career goal is to become an active physician-scientist at the direct interface between rigorous science and evidence-driven clinical innovation. More specifically, in the setting of a large research institution with an adjunct trial-oriented academic hospital, I plan on translating *in vitro, in vivo,* and *ex vivo* bench findings on clinically-relevant patient materials to therapeutic clinical trials, which I will directly develop and oversee. Tumor immunology and the specific role of immunotherapy in cancer treatment are of particular interest to me, both scientifically and clinically. Thus, in addition to completing my PhD in immunology, with a focus on tumor-specific immunologic processes, I plan on further pursing an MD with a specialization in medical oncology. I hope to use these clinical skills in my future career to establish a specialized clinical role from which I will gain first-hand knowledge of the medical needs I will target in my research, as well as provide a source for patient enrollment in therapeutic trials. Ultimately, I hope to directly transition the insights gained from both scientific and clinical approaches to hypothesis- and evidence-driven therapies for unmet medical needs in cancer diagnosis and therapy.

In the pursuit of this goal, the training encompassed by this fellowship will provide fundamental scientific understanding of concepts in immunology and cancer biology from which to base my future endeavors in cancer therapeutic development; research and professional skill development in generating hypotheses, designing rigorous experimental methods, and communicating scientific content and significance to facilitate my future career as an independent researcher; and specific knowledge of clinical skills as well as clinical trial design, regulatory processes, clinical research ethics, and data analysis to support my future research role at the interface with clinical medicine. Fundamental scientific knowledge will be gained during this training from numerous avenues, including critical literature review throughout the research period; select coursework, including courses directly addressing immunobiotherapeutics and cancer biology & therapeutics; and participation in seminars offered through the Immunology Graduate Program and the University of Pittsburgh Cancer Institute, including the Tumor Immunology Series. Development of the research and professional skills critical to my future career as an independent investigator will be accomplished by a closely-mentored research experience; presentations in numerous contexts, from lab meetings to international conferences, including meetings of the American Association of Cancer Research and the Society for Immunotherapy of Cancer; and preparing research manuscripts and grant proposals. Additionally, preparation for my future role as a translational researcher heavily involved in clinical studies will be supported during this training by the completion of a rigorous clinical curriculum; select specialized clinical research coursework, particularly through the University of Pittsburgh's Institute for Clinical Research Education; direct involvement with clinical cancer materials (Specific Aim 2); and mentorship from a sponsor intimately familiar with translational clinical trial work (see "Selection of Sponsor and Institution" section).

In aggregate, the rigorous dual degree training plan outlined by this fellowship proposal would not only add value and scope to my research and clinical pursuits, but more importantly provide concrete support to the integration of these roles and uniquely position me for a future career as a true physician-scientist involved in translational cancer research.

Activities Planned Under This Award

Percentage of Award Time in Proposed Activities				
Year	**Research**	**Coursework**	**Teaching**	**Clinical**
First	85	5	5	5
Second	95	5	0	0
Third	5	0	0	95
Fourth	5	0	0	95

Coursework planned under this award include a didactic course on Cancer Biology and Therapeutics (including immunobiotherapeutics) and a literature discussion seminar on Contemporary Topics in Immunology. These courses will complement the majority of coursework that I will have already completed by the time of this award, including core Immunology Graduate Program courses (providing knowledge of advanced immunology concepts and instruction in research design, methods, and analytic techniques), a course on Clinical and Translational Research Methods (including research ethics and biostatistics), a Professional Development course (covering grant-writing, networking, and scientific presentation and publishing), a dedicated Ethics for Medical Scientists course, and short non-credit technical courses in biologic imaging techniques offered by the University of Pittsburgh Center for Biologic Imaging. These courses will provide a formal supplement to my basic science knowledge, foster technical expertise facilitating my current and future research work, and develop skills valuable to a career in translational research, including critical literature review, presentation skills, and clinical study design skills.

Additionally, a short teaching assistant appointment for the University of Pittsburgh School of Medicine's Medical Microbiology course will provide valuable exposure to mentorship and teaching, key components of a future career at an academic medical center. I will also be participating in seminars/meetings associated with the Immunology Graduate Program (Immunology Seminar), the University of Pittsburgh Cancer Institute (specifically, the Tumor Immunology Series, the Cancer Biology & Immunology journal club, and the monthly meeting of the Cancer Immunology, Immunotherapy, and Immunoprevention Program), the Magee Womens Research Institute (monthly seminar), and the Medical Scientist Training Program (monthly seminar/workshop), which will provide the opportunity to broaden my knowledge on a diversity of topics including those related to cancer and cancer immunology, associate with potential collaborators and resources for new ideas, and receive career guidance through interaction with active physician-scientists.

Clinical activities will be comprised of a Longitudinal Clinical Clerkship (a 20 week, one half-day per week clinical rotation individually tailored to my interest in medical oncology) during the first year of the fellowship, which will provide a continued connection with clinical medicine during my research training, foster the career integration of research and clinical practice, and provide an up-to-date understanding of the medical needs that underlie translational cancer research. I plan on completing this clerkship with Dr. John Kirkwood, MD (Professor and Vice Chair for Clinical Research, Department of Medicine; Director, Melanoma Program; Chairman, Melanoma Committee of the Eastern Cooperative Oncology Group), a renowned clinician and clinician-researcher and close collaborator with our lab. This will complement the final two years of fellowship training, which will be comprised primarily of clinical work in the completion of an MD degree. During this period, however, elective research rotation months within the medical school curriculum will be used to pursue discrete clinical research projects to specifically explore cancer research from a clinically-focused perspective, providing a balanced complement to my current bench-based dissertation work. For these projects, I plan on working with our current active clinical collaborators, including Dr. Kirkwood, with whom I will have already established relationships with during my graduate research training. The clinical training proposed under this fellowship would allow me to gain the clinical competencies critical to a future career in patient-oriented biomedical investigation, including experience with the clinical monitoring of patients and the clinical implementation of trial protocols, as well as a more-comprehensive perspective on translational cancer research.

Doctoral Dissertation and Other Research Experience

- "Soluble, single-chain T cell receptor/cytokine fusion proteins for cancer immunotherapy," Altor Bioscience, Miramar, FL (6/2002-6/2003)
    - Advisor: Shari Schiavi, PhD, Senior Scientist, Altor Bioscience
    - Project Summary: Development of a fusion protein linking IL-2 with a soluble, single-chain T cell receptor construct recognizing a cancer-associated mutated p53 peptide/MHC complex, with direct application for the enhancement of targeted cytokine-based cancer immunotherapy. Worked with mammalian expression, purification, and *in vitro* activity assays of the construct.
    - Techniques: Mammalian cell transfection, cell culture, and cell staining, as well as immunoaffinity chromatography and ELISA.
    - Accomplishments: Authorship on two publications.
- "Anti-tissue factor antibodies for cardiovascular and inflammatory disease," Altor Bioscience, Miramar, FL (6/2002-6/2003)
    - Advisor: Heather Belmont, PhD, Senior Scientist, Altor Bioscience
    - Project Summary: Development of a novel, monoclonal chimeric antibody used as a tissue factor antagonist for cardiovascular and inflammatory disease. Worked to sequence the mammalian expression vector.
    - Techniques: Primer construction, PCR isolations and amplifications, sequencing reactions, and computer analysis using Sequencher and Vector NTI.
    - Accomplishments: Authorship on one publication; work helped form basis for successful filing of an FDA Investigational New Drug application for human clinical study in stable/unstable angina, acute lung injury, and acute respiratory distress syndrome.
- "Polysiloxane/silicate core-shell nanoparticle encapsulation systems," Department of Chemistry, University of Florida, Gainesville, FL (5/2004-5/2006)
    - Advisor: Randolph Duran, PhD, Associate Professor, University of Florida
    - Project Summary: Synthesis and characterization of a novel microemulsion-based core-shell nanoparticle encapsulation system for small-molecule uptake and delivery systems. Worked with surfactant synthesis optimization and capsule characterization through fluorescent dye encapsulation. Also worked to create drug and dye-loaded capsules surface-functionalized with single-chain T cell receptor constructs recognizing cancer-associated peptide/MHC complexes, by creating biotinylated and biocompatibilized poly(ethylene glycol) nanocapsules amenable to surface modification. Constructs from this project were developed for application in targeted drug delivery and imaging approaches.
    - Techniques: Dynamic light scattering, transmission electron and atomic force microscopy, UV-vis and steady-state fluorescence spectroscopy, and basic organic synthesis.
    - Accomplishments: Authorship on two publications; successful competition for national Goldwater and Beckman Foundation fellowships; oral and poster abstracts presented at several national and regional symposia.
- "Retroviral vectors in T cell gene therapy," Surgery Branch, National Cancer Institute Center for Cancer Research, National Institutes of Health, Bethesda, MD (6/2006-8/2006)
    - Advisor: Richard Morgan, PhD, Staff Scientist, National Institutes of Health
    - Project Summary: Within the context of T cell gene transfer, specifically the transduction of cancer peptide-specific T cell receptors into lymphocytes to confer targeted anti-tumor activity, worked to investigate potential tumorigenic consequences of retroviral-based T cell gene therapy. Specifically studied insertion sites of a murine leukemia virus-based vector in the genome of a human T cell clone exhibiting sustained logarithmic *ex vivo* growth in the absence of exogenous cytokine support after retroviral transduction with an IL-15 gene. Project was developed to optimize genetically-modified T cell-based cancer immunotherapy.
    - Techniques: Classical phage genomic library construction and linker-mediated and linear amplification-mediated PCR.
- "Processing and Presentation of HLA-A2-p53 Complexes by Human Papilloma Virus-infected Cancers," Department of Immunology, University of Pittsburgh, Pittsburgh, PA (6/2007-8/2007)
    - Advisor: Robert Ferris, MD, PhD, Professor, University of Pittsburgh
    - Project Summary: Characterization of the effect of the HPV-16 oncoprotein E6 on the surface expression of various p53 epitopes in E6-transduced and naturally-transformed HPV-infected head and neck squamous cell carcinoma (HNSCC) cell lines, primarily using novel epitope-specific

single-chain T cell receptor staining reagents. Project was developed to enhance targeted cancer therapies and inform strategies for augmenting adaptive immune recognition of HNSCC.
    - Techniques: Flow cytometric analysis using tetrameric single-chain T cell receptor staining reagents, chemical siRNA transfection, and adenovirus transfection.
    - Accomplishments: Abstract presented at American Medical Association Fall 2008 National Meeting.
- "Interactions between Natural Killer Cells, Dendritic Cells, and Myeloid-Derived Suppressor Cells in Human Cancer," Department of Immunology, University of Pittsburgh, Pittsburgh, PA (6/2008-8/2008; 6/2009-present); Doctoral Dissertation Project
    - Advisor: Pawel Kalinski, MD, PhD, Professor, University of Pittsburgh
    - Project Summary: Characterization of the reciprocal immunologic interactions between differentially-activated natural killer (NK) cells, dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs). In the context of cell-based cancer immunotherapy, the project focuses on elucidating the requisite signals for the acquisition and performance of desirable immune-stimulatory NK cell functions (killing of tumor cells and MDSCs, activation of DCs, and induction of productive chemokine-driven DC-effector cell interactions) and undesirable immune-suppressive NK cell functions (killing of DCs and MDSC activation). Project has direct relevance to enhancing NK cell- and DC-based cancer immunotherapies by identifying potential selective means to modify distinct NK cell activities.
    - Techniques: Isolation, culture, and/or differentiation of NK cells, T cells, DCs, and MDSCs from human peripheral blood and tumor tissue; surface and intracellular flow cytometric analysis; ELISA; ELISPOT; ^{51}Cr release and flow-based cytolytic assays; *in vitro* chemotaxis assays; RNA isolation and RT-PCR; tumor histology; and confocal and live cell microscopy.
    - Accomplishments: Authorship on one publication; oral and poster abstracts presented at several international and regional symposia.

The proposed research training plan will develop a strong scientific, technical, and professional foundation for a future career as an independent investigator in translational cancer research. The proposal provides a strong vehicle for acquiring the conceptual and technical knowledge necessary for future work in the field of cancer immunology and cancer immunotherapy, including experience with diverse cellular, protein, and genetic assays (including advanced biologic imaging techniques) as well as familiarity with the regulation, acquisition, and use of human clinical materials. The proposed research training plan further provides for the structured development of critical research thought, experimental design, ethical decision-making, and written and oral scientific communication through a closely-mentored research experience, a formal scientific and professional skill-development curriculum, and intramural and extramural interactions with leading cancer researchers and physician-scientist role-models. Please see "Training Plan" section II.C.1 for more detail.

Section II – Sponsor and Co-Sponsor Information

II.A. Research Support Available

Current Research Support		
P01 CA101944 (NIH/NCI) "Integrating NK and DC into Cancer Immunotherapy" Role: PI of Project 2	**(Lotze/Kalinski)**	**07/06/2005 – 06/30/2011** $1,243,053
R01 CA095128 (NIH/NCI) "Regulation of DC Activity by Memory and Effector $CD8^+$ T cells" Role: PI	**(Kalinski)**	**07/01/2010 – 06/30/2011** $537,066
P01 CA132714 (NIH/NCI) "Directing Tumor-Specific T cells to Tumors" Role: Overall Program PI, PI of Project 1 and Core A, Co-I on Project 3	**(Kalinski)**	**04/27/2009 – 03/31/2014** $717,288
R01 CA134633 (NIH/NCI) "Clinical Translation of 19F MRI to Visualize Cancer Immunotherapeutic Cells" Role: PI (MPI)	**(Ahrens/Kalinski)**	**05/10/2009 – 04/30/2012** $1,200,000
P50 CA121973 (NIH/NCI) "SPORE in Skin Cancer" Role: PI of Project 3	**(Kirkwood/Falo)**	**08/26/2008 – 07/31/2013** $2,300,000
P01 CA109688 (NIH/NCI) "Immune Escape of Human Cancers: Cellular Mechanisms and Countermeasures" Role: Co-I	**(Whiteside)**	**09/30/2006 – 07/31/2011** $1,071,960
Pending Research Support		
P01 CA101944 (NIH/NCI) "Integrating NK and DC into Cancer Immunotherapy" Role: Overall Program Co-PI (MPI), PI of Project 2, Co-PI of Core A	**(Lotze/Kalinski)**	**10/1/2011 – 09/30/2016** $1,091,814

II.B. Sponsor's Previous Fellows/Trainees

Past Trainees in Kalinski Lab (selected from 15 previous trainees)			
Name	**Training Period**	**Project**	**Current Position**
Je-Jung Lee, MD, PhD	2006 - 2007	DC1-based vaccines in hematologic malignancies	Associate Professor of Hematology-Oncology, Chonnam National University, Gwangju, Korea; Director, Research Center for Cancer Immunotherapy, Chonnam National University Hwasun Hospital, Jeollanamdo, Korea
Young-Ik Son, MD, PhD	2000 - 2001	1) IL-12 production by $CD8a^+$ and $CD8a^-$ DC in mice; 2) synergistic anti-tumor activities of IL-12 and IL-18	Associate Professor, Department of Otorhinolaryngology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea
Yutaro Nakamura, MD, PhD	2003 - 2005	Helper function of mouse $CD8^+$ T cells	Assistant Professor, Second Dept Internal Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan
Adam Giermasz, MD	2001 - 2005	Polarized dendritic cells as a tool to induce anti-cancer immunity	Fellow, Division of Hematology/Oncology, Department of Medicine, University of California San Francisco
Robbie Mailliard, PhD	2002 - 2006	Helper role of $CD8^+$ T cells and NK cells in the induction of DC-mediated Type-1 immunity.	Research Assistant Professor, Department of Infectious Disease and Microbiology, University of Pittsburgh

II.C. Training Plan, Environment, Research Facilities

II.C.1. Training Plan

Graduate Coursework:

Jeff has and will continue to benefit from the extensive curricular resources of the University of Pittsburgh to develop both an enhanced conceptual knowledge of immunology and cancer biology as well as specific practical knowledge in research design and techniques. By the start of this fellowship period, Jeff will have completed the vast majority of his Immunology Graduate Program coursework. This includes advanced didactic courses on Comprehensive Immunology (examining advanced immunologic concepts in immune cell

development, recognition, signaling, and interactions) and on Immunology in Health and Disease (examining immune paradigms in human health, including cancer immunology). Jeff will have also completed a course on the Experimental Basis of Immunology, an intensive didactic/workshop course analyzing contemporary experimental methods. Furthermore, Jeff will have also completed an elective in Clinical and Translational Research Methods, offered through the Institute for Clinical Research Education, which will facilitate his understanding of trial-oriented research, including study design, ethics, and statistical analysis. These skills will be very relevant to his future role as an independent investigator closely involved with clinical studies.

During the award period, Jeff will complement this completed coursework with a dedicated course on Cancer Biology & Therapeutics, which will explore in detail the current and emerging paradigms and treatment approaches in cancer, including a block dedicated to cancer immunotherapy. This course will provide a sound didactic basis for a future career in developing translational cancer immunotherapies. As relevant, Jeff will also take unique advantage of short, non-credit courses in microscopic imaging techniques offered through the renowned University of Pittsburgh Center for Biologic Imaging, which will facilitate both the completion of his fellowship project as well as establish a foundation in imaging skills highly relevant to investigation in this field.

Research Experience:

Throughout this fellowship, Jeff will learn how to function as an independent investigator while concurrently building a strong base of scientific and technical knowledge for a future career in translational cancer research. Completion of his research proposal will provide a strong vehicle for acquiring essential knowledge of contemporary techniques in immunologic research, including a diverse range of genetic, protein, and cellular assays. Jeff will also gain a strong working knowledge of biologic imaging techniques, supported by the mentorship and expertise of Dr. Simon Watkins, PhD (a member of Jeff's thesis committee and Director of the Center for Biologic Imaging) and members of his group. His research proposal will also provide an avenue for understanding the acquisition and use of human clinical materials, essential to translational cancer research. His development of critical research thought, experimental design, and scientific communication (both in manuscripts and poster and oral presentations) will continue to be developed through multiple avenues described in detail below, including close and frequent interactions with myself and interactions with his thesis committee, members of the broader University of Pittsburgh environment, and the cancer research community.

Intramural Meetings and Seminars:

I meet with Jeff weekly on an individual basis and will continue to do so throughout his training. In these meetings we discuss in detail his ongoing and future research, including technical approaches, analysis of primary data, and conceptual implications. These meetings also regularly focus on professional development. For instance, we often discuss meeting abstract and manuscript drafts to help focus his work, assess future experiments, and gain perspective on how his work might be evaluated by outside reviewers. We also discuss opportunities to present his work to diverse audiences, whether at institutional meetings or larger conferences. Jeff will also benefit from our weekly lab meetings, which broaden his mentorship and refinement of scientific ideas, particularly during presentation of his work to the group approximately every two months.

Jeff completed his PhD Comprehensive Examination in December 2010 and has since formed and met with his dissertation committee, which consists of:

- Pawel Kalinski, MD, PhD (Committee chair and dissertation advisor; Professor of Surgery, Immunology, and Infectious Disease & Microbiology; Director of Research, Division of Surgical Oncology)
- Robert Edwards, MD (Professor and Executive Vice Chair of Obstetrics, Gynecology, and Reproductive Sciences; Director, Ovarian Cancer Center of Excellence)
- Michael Lotze, MD (Professor of Surgery, Immunology, and Bioengineering; Vice Chair of Research, Department of Surgery; Director of Strategic Partnerships, University of Pittsburgh Cancer Institute)
- Walter Storkus, PhD (Professor of Dermatology and Immunology)
- Simon Watkins, PhD (Professor and Vice Chair of Cell Biology & Physiology; Director, Center for Biologic Imaging)
- Per Basse, MD, PhD (Associate Professor of Immunology)

Jeff's committee members are experts in their respective fields and collectively offer an incredible resource for basic and translational scientific knowledge (including NK cell and myeloid cell biology), clinical and clinical trial experience, and technical expertise (including advanced biologic imaging) that will contribute strongly to Jeff's dissertation work and development as a translational scientist. Meetings with this entire group will occur formally every 6 months, in which his dissertation progress will be reviewed in detail. More frequent meetings with individual members will be available as needed for technical, scientific, and/or career advice.

Jeff will also benefit from semi-annual formal and more frequent informal meetings with his MSTP Career

Advisor, Dr. Gary Silverman, MD, PhD (Professor of Pediatrics and Cell Biology & Physiology; Chief, UPMC Newborn Medicine; Director, Neonatal-Perinatal Training Program). Dr. Silverman offers particular expertise in mentoring students on research/clinical balance, MD/PhD training, and physician-scientist career milestones.

In addition, his participation and presentation at numerous intramural seminars will provide critical and timely exposure to contemporary topics in the field and enhanced interactions with peers and experts, supporting the development of his collaborative skills. These seminars include the Immunology Department seminar, the University of Pittsburgh Cancer Institute (UPCI) Tumor Immunology Series, the UPCI Cancer Biology & Immunology journal club, and the Magee-Womens Research Institute monthly seminar. Additionally, Jeff will participate in the UPCI's monthly Cancer Immunology, Immunotherapy, and Immunoprevention Program meeting, which will provide first-hand experience with investigative integration across multiple groups in both research and clinical spheres. Jeff will also present yearly at the annual retreats of the UPCI, which will continue to foster his scientific communication skills as well as scientific interactions at an institutional level.

Extramural Interactions:

Jeff's training will heavily incorporate interactions outside the University of Pittsburgh, which will allow the diversification of his conceptual exposures, refine his oral and written communication skills, and facilitate connections with individuals and institutions highly relevant to his future goal of becoming an independent researcher in the cancer immunotherapy field. Specifically, his presentations at the meetings of the American Association of Cancer Research and the Society for Immunotherapy of Cancer (which I organized this past year) would place him in close contact with the foremost research and researchers in cancer immunology and immunotherapy, developing his intellectual and collaborative skill directly as it relates to cancer (scientifically and clinically) and cancer immunotherapy.

Clinical Experiences:

Jeff will be working in numerous clinical capacities during the training period, which will help develop the clinical skills necessary for a future career as a physician-scientist with direct patient and clinical trial involvement. The Longitudinal Clinical Clerkships (LCCs), which represent 20 week clinical rotations for one half-day per week, will be specifically focused on medical oncology, in concordance with Jeff's clinical and career interests. This will provide highly valuable exposure to clinical fields directly related to and impacted by his concurrent research, enhancing the research/clinical integration important to his planned career as a translational investigator. He has already completed one such LCC with Dr. Suzanne Lentzsch, MD, PhD (Assistant Professor of Medicine with the Division of Hematology/Oncology; Clinical Director of the Multiple Myeloma Program), for which he received outstanding performance evaluations. Dr. Lentzsch is also one of our research collaborators, providing a unique opportunity for Jeff to directly experience the integration between research and clinical practice. During this fellowship period, Jeff will complete another LCC with Dr. John Kirkwood, MD (Professor and Vice Chair for Clinical Research, Department of Medicine; Director, Melanoma Program; Chairman, Melanoma Committee of the Eastern Cooperative Oncology Group). Dr. Kirkwood is a highly accomplished researcher and clinician, as well as a close collaborator with our lab and a co-author on Jeff's most recent publication. He would provide the ideal mentor for integrating Jeff's research and clinical interests, and facilitate the development of his clinical cancer research skills.

This clinical experience during the research portion of the fellowship will subsequently transition to majority-time clinical work in completion of his medical degree, although his continued involvement in our lab's data analysis and critical review of lab manuscripts will maintain the connection to his thesis training. The relationship developed with Dr. Kirkwood during his LCC experience will also provide the foundation for a planned clinical research experience during Jeff's 3rd and 4th year of medical school, in which he will participate in discrete clinical research projects during an elective research rotation. This will allow him to gain a better understanding of research from a clinically-focused perspective, as a supplement to the laboratory research experience gained from his thesis work.

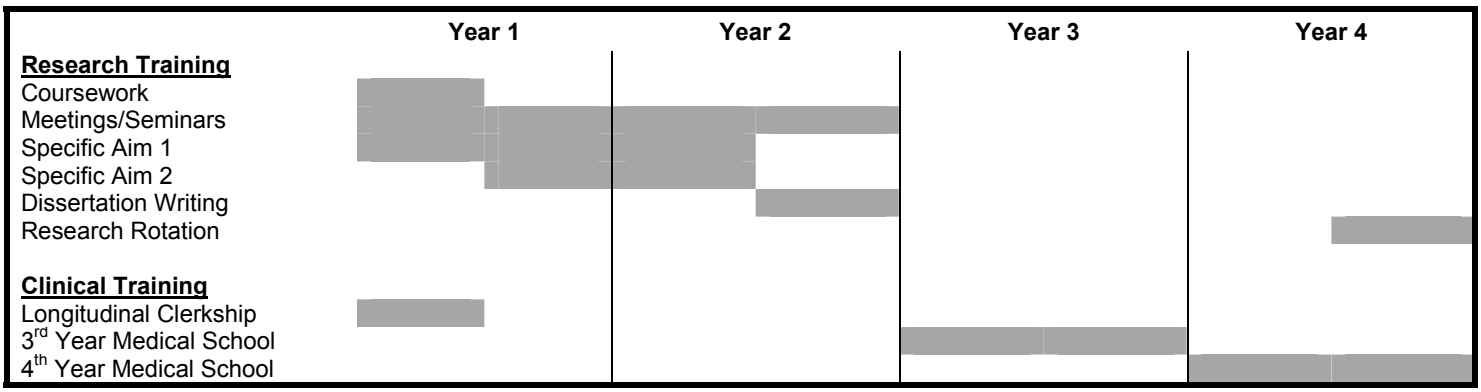
Professional Development:

In addition to the already-completed MSTP Professional Development course, which provided formal instruction on scientific writing, presenting, and networking, Jeff will participate in various activities designed specifically to enhance his professional skills. His oral communication will be developed through his participation in the meetings and seminars described above, as well as regular presentation of his data, critique of other projects, and literature presentation within our weekly lab meetings. His written skills will be developed throughout the research and clinical training period via his personal manuscript preparation, with input from me and other lab members and collaborators, as well as through his critical review of manuscripts and grant applications prepared by others. Finally, mentorship, teaching, and leadership skills will be

developed through a formal teaching assistantship for the School of Medicine Medical Microbiology course.

Ethics Training:

Training in the responsible conduct of research and clinical care is a key aspect of the MSTP dual-degree program at the University of Pittsburgh. This training comprises multiple formats integrated longitudinally across both medical and graduate phases. Jeff will have already taken a semester-long didactic/workshop course on Ethics, Law, and Professionalism provided as part of the medical school curriculum as well as an additional month-long didactic/workshop course on Ethics for Medical Scientists through the MSTP program. As relevant to Jeff's work, he has also completed self-directed internet-based training modules on research integrity, human subject research in biomedical science, conflict of interest, and HIPAA privacy requirements for researchers. Jeff further participates in ethics-based seminars twice a year through the MSTP program, and will continue to participate in these seminars for the duration of his training. Please see the section on the "Responsible Conduct of Research" for a detailed description of all of these activities.



II.C.2. Environment

Kalinski Laboratory:

My laboratory is highly-experienced and well-suited for training independent translational investigators with clinically-relevant roles, as evidenced by my past trainees (see section II.B), which include several MD/PhDs. Additionally, as a member of the Interdisciplinary Biomedical Graduate Program of the School of Medicine and of the Graduate Program of the Graduate School of Public Health, I am or have been a member of eight comprehensive exam committees and a member of nine PhD thesis committees. I am also a member of the Junior Faculty Mentoring Committee of the UPCI, developed in order to support the transition of our junior faculty members to tenure-track positions. I also currently serve as a Co-Investigator on a T32 training grant specifically focused on training fellows in research relevant to oncology and the biological therapy of cancer.

Jeff will benefit from this experience during regularly scheduled, one-on-one weekly meetings with me to discuss the progress of his projects, abstract and manuscript preparation, and other career and professional development issues (see "Training Plan" section II.C.1 for more detail). Lab members can further consult with me at any time on an ad hoc basis (my office is adjacent to the lab). The training potential of my group is enhanced by the availability of a senior group member and research manager, Dr. Eva Wieckowski, with vast experience in the field of cancer immunology and immunotherapy. My research group also benefits from a faculty Research Instructor, Dr. Ravikumar Muthuswamy, with considerable experience in tumor immunology, especially as it relates to clinical tumor materials (directly relevant to Jeff's proposal). The lab training environment is also supported by two postdoctoral fellows with considerable experience in imaging and other immunologic assays, including as they specifically relate to myeloid-derived suppressor cells from ovarian cancer (also directly relevant to his proposal). Jeff may also benefit from practical advice and an exchange of ideas with a senior graduate student in the lab. All lab members also regularly present their projects to the entire group (every 2 months) during our weekly group meetings, receiving valuable scientific feedback.

University of Pittsburgh, Division of Surgical Oncology of the University of Pittsburgh Cancer Institute (UPCI), and the Magee-Womens Hospital (MWH)/Magee-Womens Research Institute (MWRI):

The research and training potential of my lab is enhanced by ongoing interactions within the extremely rich scientific and training context of the University of Pittsburgh, the UPCI, and the MWH/MWRI.

The University of Pittsburgh is widely recognized as a leading academic institution in the US. Consistently within the top 10 recipients of overall NIH funding, the focus of the university's research efforts on medical science and biotechnology places our group in the center of the university's research and training activities.

The commitment of the university to the research and career development of pre-doctoral MD/PhD trainees is emphasized by the strength of the MSTP, which enjoys strong funding support from both the NIH and the School of Medicine and the resources necessary to develop and support a unique, highly-integrated curricular training program tailored specifically for combined medical and graduate research training.

The University of Pittsburgh Cancer Institute (UPCI) is the only NCI-designated Comprehensive Cancer Center in western Pennsylvania. In 2010, the UPCI received almost $170 million in research funding and was ranked 11th nationally in funding from the NCI, demonstrating its strength in biomedical cancer research and the quality of the colleagues and mentors Jeff will have access to during his training. The UPCI is housed in the Hillman Cancer Center, which brings under one roof diverse clinical, research, and educational/training facilities. This will facilitate Jeff's interactions with other research groups, networking with both research and clinical faculty, and acquisition of clinical materials for his studies. The large number of cancer-oriented scientists, research programs, and visiting scientists results in a high number of research seminars and conferences related to cancer, which Jeff has access to, as well as an extremely robust UPCI annual retreat.

I serve as the Direct of Research for the Division of Surgical Oncology within the UPCI. Intensive interactions between the scientific and clinical faculty of the Division facilitates joint development and clinical introduction of new cancer therapies, with a specific focus on cancer immunotherapy. Jeff will directly benefit from being situated within this Division through exposure to this translational cancer research integration. Jeff will also benefit from our close association with Magee-Womens Hospital (MWH), recognized as a National Center of Excellence in Women's Health and among the top 12 hospitals nationwide for gynecological care, and the Magee-Womens Research Institute (MWRI), the nation's first research center devoted exclusively to health conditions affecting women and their infants. Particularly relevant to Jeff's proposal is the strength of MWH's Division of Gynecologic Oncology, which sees over 100 newly-diagnosed ovarian cancers yearly. Facilitated through his interactions with Dr. Robert Edwards, MD (Director of the Ovarian Cancer Center of Excellence, our close research collaborator, and a member of Jeff's thesis committee), Jeff will have access to the considerable expertise of this division and to patient materials necessary for the completion of his proposal.

II.C.3. Research Facilities and Equipment

My lab occupies approximately 1,200 sq. ft. of space on the first floor of the Hillman Cancer Center Building. The lab is fully equipped for cellular immunology research, with: 4 laminar flow hoods, 4 CO_2 incubators, 3 adjustable speed/temperature cell centrifuges, 3 inverted microscopes, a PCR cycler (Taqman), water bath incubators, a histologic microscope, 2 Eppendorf centrifuges, 3 scales, 2 -80°C freezers, 2 -20°C freezers, 2 -4°C refrigerators, other minor laboratory equipment, and the necessary software and hardware for data analysis. Shared facilities in immediate proximity provide equipment for ELISA and ELISPOT analysis, liquid nitrogen cryopreservation tanks, gamma and beta counters, HPLC equipment, histology cryostats, cell harvesters, and high speed- and ultra-centrifuges. Space and fixtures include an additional molecular biology room with fume hood, molecular histopathology room, dark room, and walk-in cold room. As a part of the UPCI, Jeff will have further full access to its extensive core facilities (the most relevant below):

- Flow and Imaging Cytometry Core (adjacent to the lab): contains equipment for 4- and 12-color flow cytometry, cell-sorting, and a Cellomics Arrayscan HCS reader (Thermo Scientific)
- Biologic Imaging Satellite Facility (adjacent to the lab; led by Dr. Per Basse, MD, PhD, a member of Jeff's thesis committee): contains multiple light microscopes, a temperature- and CO_2-controlled time-lapse imaging microscope, confocal and two-photon microscopes, and microtomes
- Luminex Core: provides a Luminex Bio-Plex workstation for cytokine/chemokine profile analysis
- Tissue and Research Pathology Services: provides centralized tissue and biological specimen procurement, research histology, annotated clinical data, and tissue microarray services

Other core facilities include a Viral Vector Core, Peptide Synthesis Facility, Proteomics and Mass Spec Core, Immunologic Monitoring Facility, and Biostatistics Core (see "Equipment" section). Jeff will have further access to the Center for Biologic Imaging, which encompasses 17 confocal microscopes, 2 TEM microscopes, 1 SEM microscope, 17 additional assorted microscopes for various applications, extensive imaging processing and analysis hardware and software, and biologic imaging expertise of dedicated staff. Additional intellectual resources include clinical research expertise through the Clinical and Translational Science Institute.

II.D. Number of Fellows/Trainees to be Supervised

I am currently supervising two graduate students and two postdoctoral fellows.

II.E. Applicant's Qualifications and Potential for a Research Career

As an investigator with a career-long involvement in translational cancer research, I have come to appreciate unique qualities necessary for success in the demanding research environment at the junction

between the lab and the clinic. These qualities include intellectual curiosity, dedication, and originality, but also the practical insight, collaborative skill, and communication necessary to translate scientific knowledge into practical application. Having had the opportunity to mentor several clinical-oriented research trainees in my career, I know these qualities are actively embodied and cultivated only by certain driven individuals. I believe Jeff is one such individual, and I believe that, with the help of this fellowship, he has the outstanding potential to develop into a highly successful, independent investigator focused on translational cancer research.

I have known Jeff since spring 2008, when he applied for a summer rotation in my lab. I accepted him as a rotation student being intrigued by both his impressive CV (already at that stage he was a co-author of several papers and the first author of several research abstracts) and by his intellectual curiosity for how the immune system works and how it can be modulated to help patients with cancer. Over the summer of 2008 and since the beginning of his PhD work in the summer of 2009, I have had ample opportunity to evaluate his research ability and potential for becoming an independent researcher. From the very first weeks of our interaction, I was impressed with his scientific creativity and enthusiasm and his ability to formulate hypotheses, design experiments, perform them, and interpret the data. He also displayed impressive initiative in advancing collaborative studies, understanding the value of joining expertise toward a common question. For instance, he worked with the other graduate student in the lab to develop parallel studies comparing natural killer and cytotoxic T cell interactions with dendritic cells and tumor cells. This has also translated to his interactions with others outside of our lab. For instance, he has developed an arm of his proposal through discussions with another mentor, Dr. Michael Lotze, to combine our expertise in NK and DC biology with their expertise in cell death mechanisms to investigate how different modes of NK cell activation may affect anti-tumor responses.

Of particular interest to me was (and continues to be) Jeff's interest in NK cell immune-regulatory interactions and the translational therapeutic significance of these findings for patients with cancer. His dedication to becoming an expert in this field is unmistakable, manifested in his continued rigorous survey of the available scientific literature. This is supported by his incredible work ethic, his practical problem-solving abilities when obstacles arise, and his very receptive use of guidance and mentorship from multiple sources. These attributes have contributed to a very productive start to his PhD, highlighted by a first author manuscript and presentation of his work at international symposia. He has also prepared a strong thesis proposal, which he successfully defended as part of the Immunology Department's Comprehensive Examination more than half a year earlier than other students in his same PhD matriculating class. I was extremely impressed by the research proposals Jeff independently prepared for his thesis and this application, which indicates his exceptional potential for original research thought. Jeff has also demonstrated the key ability to be able to communicate not only his scientific findings, but also the scientific and clinical significance of his work, which is vital to a successful career in translational research. For instance, he was recently selected as a Richard L. Simmons Award recipient as a top oral presenter in the Department of Surgery Annual Research Symposium, making his work relevant to a challenging audience of diverse scientific and clinical backgrounds.

Taking into account Jeff's scientific interests, his impressive past experience with several laboratories (including with the NIH), and his documented record of success (including among many others, as College of Liberal Arts and Sciences Valedictorian at the University of Florida and selection for the national Goldwater and Beckman Fellowships, an NCI Cancer Research Training Award, and an Individual Pre-Doctoral Fellowship from the Clinical and Translational Science Institute based on proposals of his own design), I have very high expectations for his future success as a physician-scientist. I believe that his scientific interests and his career goals are highly compatible with my research group, which translates findings on NK cell and CTL interactions with dendritic cells to develop new cancer immunotherapies. Since several of these approaches involve close collaboration with physicians and physician-scientists, they will provide the patient samples relevant to his proposal and the opportunity for Jeff to be exposed to the logistic and regulatory issues related to the conduct of clinical studies. Already, Jeff has fully utilized these collaborations to refine his scientific and clinical interests. For instance, he initiated a Hematology/Oncology clinical clerkship with our collaborators, Dr. Suzanne Lentzsch, MD, PhD (Clinical Director of the Multiple Myeloma Program) and Dr. Markus Mapara, MD, PhD (Director of the Hematopoietic Stem Cell Transplantation Program), for which he earned outstanding 'honors' evaluations for his clinical performance while simultaneously maintaining his lab productivity and developing some very interesting ideas for future collaborative studies. This truly demonstrates Jeff's capability and potential for synergistically integrating his research and clinical pursuits.

Based on the particularly strong record of Jeff's past achievements and my own personal experience as his research supervisor, I am looking forward to continuing my role as Jeff's mentor, and believe that with the help of the currently described training, he will become an exceptional physician-scientist set for an outstanding career in translational cancer research.