January 30th, 2023

Dear Steering Committee,

It is with great pleasure that we submit our proposal titled “Spatial transcriptomic profiling of the sentinel lymph node to identify molecular determinants of tumor recurrence” for consideration for the Synergy Grant.

This proposal represents a new collaboration across Cancer Biology (Dr. Huang), Surgical Oncology (Dr. Karakousis), and Biostatistics (Dr. Li).

Sincerely,

Alexander Huang, MD
Assistant Professor of Medicine
Department of Cancer Biology
Abramson Cancer Center
University of Pennsylvania

Mingyao Li, Ph.D
Professor Biostatistics
Biostatistics and Epidemiology
University of Pennsylvania

Giorgos Karakousis, MD
Professor Surgery
Hospital of the University of Pennsylvania
Significance: Adjuvant anti-PD-1 therapy (αPD-1) is FDA approved for Stage III melanoma patients with lymph node (LN) metastasis. However, only a subset of patients benefit from adjuvant αPD-1, with an absolute benefit ranging from 10% - 20% in recurrence-free survival, depending on the stage grouping (Stage III A-D). Indeed, a significant proportion of patients are cured with surgical resection alone, including >80% of Stage IIIA patients. There is currently no way to gauge who would benefit from adjuvant immune checkpoint blockade. Although Stage III melanoma patients may be prescribed adjuvant αPD-1, only a subset will directly benefit, but all are exposed to the risk of immune toxicity. Thus, there is an urgent need to risk-stratify patients to understand who would have the highest risk of recurrence and why. This proposal is focused on understanding the immunological consequences of early metastasis and identifying candidate predictive markers of recurrence in Stage III melanoma.

The sentinel lymph node (SLN) biopsy is commonly performed for its prognostic and staging utility. The SLN is identified by using a set of dyes injected intradermally at the site of the primary melanoma and identifies the first node(s) of lymphatic drainage. As the most common first site of metastasis, the SLN represents the earliest interaction between adaptive immunity and metastatic disease. Thus, the SLN biopsy provides a unique opportunity to understand the early immunologic response to metastatic cancer cells. Indeed, the immunogenicity of the tumor, or in other words the ability of the tumor to induce an immune response, is critical for the efficacy of immunotherapy, including αPD-1. We and others, have demonstrated that cellular and molecular signatures of T cell responses in the tumor are predictive of clinical benefit to αPD-1 in Stage IV and Stage III (B-D) disease. We hypothesize that the immunogenicity of the tumor is imprinted even earlier, at the time of initial micro-metastasis (Stage IIIA), which can inform on the risk of recurrence after surgical resection. We leverage the SLN biopsy to identify the initial cluster of micro-metastatic melanoma cells and use spatial transcriptomics to identify tumor-intrinsic and extrinsic mechanisms or metastasis, and potential molecular predictors of tumor recurrence.

Innovation:

1. Sentinel lymph nodes as the initial site of anti-tumor immunity. As the location of initial priming of anti-tumor immunity, the initial micro-metastasis shapes the immunogenicity of the cancer, and likely determines immune protection versus tumor progression. While SLN evaluation has been primarily used clinically to assess for micro-metastatic disease, we apply advanced molecular approaches to the SLN to understand the mechanisms of early metastasis and identify candidate biomarkers for disease recurrence.

2. Spatial transcriptomics offer novel biologic insights. The cellular and molecular mechanisms underlying the initial priming of anti-tumor immunity in humans is virtually unknown. This knowledge is limited by the ability to identify and profile the initial interactions of metastatic cells with immune cells in the draining lymph node of humans. We apply cutting-edge spatial transcriptomics to the SLN to identify early metastatic cells and generate a “spatial molecular atlas of early metastasis”, allowing for the identification of cell types and molecular pathways important in early immune control and tumor progression.

3. Novel analytic approaches. The full potential of spatial transcriptomics can only be realized when paired with innovative analytic approaches. We develop advanced machine learning algorithms to characterize spatial gene expression patterns with super-resolution and study how the spatial gene expression changes are associated with early metastasis and recurrence. These algorithms will be broadly applicable to other human studies.

Research Strategy: Cancer cells influence immune cells via a number of factors including chemokines, interleukins, hormones, or direct antigen-presentation. Thus, we hypothesize that the initial cluster of metastatic cancer cells imprint distinct molecular signatures that extends from the cancer metastasis across the SLN. Indeed, targeted gene-expression profiling identified an enrichment of T cell exhaustion-related genes such as CD200 and TIGIT in the SLN of patients who had microscopic nodal disease (SLN+, Stage IIIA) (Fig 1), compared to lymph nodes without metastatic disease (SLN-), indicating that an exhaustion signature may be one of many molecular signatures that are imprinted. We will

Figure 1: Sentinel lymph node metastasis associated with exhaustion gene signature: 800-gene Nanostring profiling was performed on SLN of 10 melanoma patients (5 SLN- and 5 SLN+). Composite exhaustion gene score was calculated as the median of 7 exhaustion-related genes.
perform spatial transcriptomics on 4 SLN- (non-metastatic LNs) and 16 SLN+ melanoma samples that are readily available, to identify the immune signatures that are imprinted on the SLN by micro-metastasis, and signatures that are associated with recurrence after surgery.

**Aim 1: Generate a molecular atlas of human lymph node in melanoma patients.** The goal of this Aim is to perform super-resolution molecular characterization of lymph nodes without metastatic disease. In order to understand molecular changes in the disease setting, we first need to have an accurate molecular understanding of uninvolved LN. 10x Visium spatial transcriptomics uses spatially barcoded oligonucleotides and allows for the generation of a whole-transcriptome molecular atlas of FFPE tissue at 55μM resolution. We will perform Visium on four SLN- samples. Since Visium does not have single-cell resolution, we will apply iSTAR, a machine learning method that the Li lab is developing to enhance whole transcriptome gene expression to 16um×16um superpixel resolution, which approaches single-cell resolution. iSTAR enhances gene expression resolution by leveraging information provided by a companion histology image (Fig. 2A). Our preliminary results indicate that iSTAR can enhance Visium gene expression resolution and resolve a focus of ductal carcinoma in situ (DCIS) with high accuracy, as compared to the Xenium targeted single-cell approach (Fig. 2B). We will infer cell type identity for iSTAR-enhanced data using ItClust11, that transfers cell type information from single-cell RNA-seq data from the Immunological Genome Project12. Results from this analysis will reveal the spatial distribution of different cell types in the tissue. We will perform graph neural network (GNN) analyses13 to model global neighborhood structure (e.g. germinal centers) providing information about the microenvironment of each cell. Aim 1 will provide a spatial molecular atlas which can help us understand the spatial organization of lymph node and the cellular niche structures in different regions.

**Aim 2: Understand the molecular changes associated with early metastasis and recurrence.** The goal of this Aim is to identify molecular features associated with metastasis and tumor recurrence. We will perform Visium on 16 SLN+ from Stage IIIA melanoma patients (8 with recurrence, and 8 without recurrence), matched for age, gender, and primary tumor characteristics. To generate molecular maps for each SLN+, we will perform iSTAR and GNN analyses, similar to Aim 1. First, to assess the impact of metastasis on normal cellular neighborhoods, we will examine whether the local neighborhood represented by GNN embeddings are different between the SLN- (Aim 1) and SLN+ samples. We will first identify and quantify the disruption of metastasis on normal neighborhoods by comparing the distributions between the GNN embeddings of SLN- versus SLN+ samples. To determine what cell types are modulated by metastasis, we will use ItClust, as in Aim 1, to compare cell type composition between SLN- and SLN+ groups. This analysis will detect new cell types/states that are present in the SLN+ group. Since the spatial location for each cell type is known, results from these analyses will inform which compartments in the lymph nodes are disrupted by early metastasis and cell types that are involved. In involved compartments, we will perform differential gene and pathway analyses, to identify molecular pathways that are associated with metastatic disease, including those that are tumor-intrinsic and immune-mediated. Finally, we apply these same analyses: i) neighborhood disruption, ii) cell type changes, and iii) molecular pathway analyses, to compare between SLN+ samples with recurrence and no recurrence. Aim 2 will define the cellular and molecular impact of early metastasis on normal LN and features that are associated with recurrence after surgery.

**Impact:** We assembled a new collaborative team across Surgical Oncology, Cancer Biology, and Biostatistics with expertise in clinical melanoma, human immunology, spatial transcriptomics, and computational approach. These studies will provide unique knowledge of the initial tumor cell-immune interaction in human melanoma, including cell types recruited and molecular circuits engaged, and in particular those that are associated with recurrence after surgery. These same spatial approaches will be applied to study the effect of αPD-1 on micrometastatic LNs in a neoadjuvant melanoma trial (PI Karakousis), serving as the foundation for a multi-PI R01.
Reference


BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: HUANG, Alexander C.

eRA COMMONS USER NAME (credential, e.g., agency login): TCSWARBEAR

POSITION TITLE: Assistant Professor, Hematology/Oncology

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

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>Completion Date MM/YYYY</th>
<th>FIELD OF STUDY</th>
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<tr>
<td>John Hopkins University</td>
<td>B.S.</td>
<td>05/2005</td>
<td>Biomedical Engineering</td>
</tr>
<tr>
<td>Mount Sinai School of Medicine</td>
<td>M.D.</td>
<td>05/2010</td>
<td>Medicine</td>
</tr>
<tr>
<td>Washington University School of Medicine</td>
<td>Resident</td>
<td>06/2013</td>
<td>Internal Medicine</td>
</tr>
<tr>
<td>University of Pennsylvania</td>
<td>Fellowship</td>
<td>06/2017</td>
<td>Hematology/Oncology</td>
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A. PERSONAL STATEMENT

I am a physician scientist with clinical training in oncology and research expertise in immunology. My research program focuses on translational cancer immunology research, taking advantage of innovative clinical trials to 1) understand mechanisms of clinical response versus resistance, and 2) identify targets for novel immunotherapies in cancer.

My most significant scientific contribution is defining the early pharmacodynamic immune effects of PD-1 blockade in human cancer. I identified for the first time an immune response to PD-1 blockade in the blood of melanoma patients. This immune response peaked at 3 weeks after the first dose of anti-PD-1 therapy (αPD-1), and the immunologically responding cells were phenotypically and transcriptionally consistent with exhausted CD8 T cells that have been reinvigorated (Huang et al, Nature 2017). Based on these results, I designed and led a translational clinical trial of neoadjuvant PD-1 blockade where patients underwent a tumor biopsy, received a single dose of αPD-1, and returned at 3 weeks for a complete surgical resection. This trial was a collaborative effort across six distinct clinical and basic science groups that allowed us to obtain paired blood and tumor tissue at baseline and at the time of peak immune response. Indeed, 30% of patients had robust intra-tumoral immune responses and complete or near-complete eradication of their tumor. Moreover, these patients uniformly remained disease-free, while the remainder of the cohort had a poor prognosis (Huang and Orlowski et al, Nature Med 2019). These studies demonstrated that the immune activity of PD-1 blockade was rapid, and that immunologic and clinical responses were determined after a single dose. The success of these two projects transformed the translational research landscape at Penn, catalyzing numerous multidisciplinary translational research groups.

Since joining the faculty at the University of Pennsylvania in July 2020, I have continued to partner with clinical trialists, pathologists and surgeons to initiate neoadjuvant clinical trials, including a new study of neoadjuvant nivolumab in melanoma (NCT4013854) as well as neoadjuvant studies in renal cell and Merkel cell carcinomas. I have also developed flow cytometric, transcriptional, and computational approaches to study the cellular and molecular mechanisms of immunotherapies in the human system. During the COVID-19 pandemic, I leveraged these skills to lead multi-institution translational studies on how CD8 T cells compensate for defective humoral immunity in hematologic cancer patients with COVID-19, which led to senior author publications (Bange and Han et al, Nature Med 2021; Lyodovyk, Kim, and Qualls et al, Cancer Cell 2022).

My research group now focuses on the concept of precision immuno-oncology, where we measure the immune responses of cancer patients with greater precision. We leverage single-cell sequencing, and spatial transcriptomic approaches to characterize the transcriptional state of T cells in specific spatial...
regions. We apply these approaches to understand the early immunologic changes in in the sentinel lymph node of melanoma patients.

Ongoing projects I would like to highlight include

**R01CA273018 (Huang, A)** 9/1/22-8/31/27
NIH/NCI
Role of progenitor exhausted CD8 T cells and the progenitor niche in anti-PD-1 efficacy
We leverage flow cytometric, single-cell sequencing, and spatial transcriptomic approaches to understand the impact of anti-PD-1 on progenitor exhausted CD8 T cells and the importance of the progenitor niche in sustaining progenitor-exhausted CD8 T cells.
Role: PI

Papers that I would like to highlight, demonstrating my expertise in human cancer immunology.


B. POSITIONS AND HONORS

**Positions and Employment**

<table>
<thead>
<tr>
<th>Year</th>
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<tr>
<td>2020</td>
<td>Assistant Professor of Medicine, University of Pennsylvania</td>
</tr>
<tr>
<td>2017 - 2020</td>
<td>Instructor of Medicine, Hematology/Oncology, University of Pennsylvania</td>
</tr>
<tr>
<td>2013 - 2017</td>
<td>Fellow, Hematology Oncology, University of Pennsylvania, Philadelphia, PA</td>
</tr>
<tr>
<td>2010 - 2013</td>
<td>Resident, Internal Medicine, Washington University, Saint Louis, MO</td>
</tr>
<tr>
<td>2008 - 2009</td>
<td>Doris Duke Research Fellowship, Washington University, Saint Louis, MO</td>
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**Licenses and Certifications**

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<td>2013</td>
<td>Current Board Certification, American Board of Internal Medicine</td>
</tr>
<tr>
<td>2013</td>
<td>Current Licensure, Medical Physician and Surgeon, State of Pennsylvania</td>
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Honors and Awards
2022    Pew Stewart Scholars Award
2022    Melanoma Research Foundation Young Investigator Team Award
2021    WW Smith Charitable Trust Award
2021    Damon Runyon Clinical Investigator Award
2021    Doris Duke Clinical Scientist Development Award
2019    Edward Holmes Award for Best Early Career Faculty Abstract
2017    Parker Institute Bridge Scholar Award
2017    Austrian Award for Basic Research as a Fellow
2013    Gregory J Gurtner Translational Research Award
2011    Austrian Award for Basic Research as a Fellow
2009    American Association of Allergy, Asthma, and Immunology Chrysalis Award

C. CONTRIBUTIONS TO SCIENCE
1. TLR agonists as an adjuvant to human dendritic cell vaccine in melanoma.
   Dendritic cells are considered the most potent of antigen presenting cells, and critical for successful activation of T cell immunity. Yet the use of dendritic cell (DC) vaccines in cancer has demonstrated limited clinical success. One factor that may contribute to this is the use of immature or incompletely matured dendritic cells with low production of IL12 - a key signal for CD8 T cell activation. In Gerry Linette’s lab, we initiated a clinical trial in melanoma patients using a DC vaccine matured with CD40L and IFNγ. We found that a subset of patients had DCs defective of IL12 production, which correlated with clinical progression. To test how to reverse this IL12 defect, I studied the use of TLR agonists as an adjuvant to our standard DC vaccine matured with CD40L and IFNγ. I found that the addition of the TLR3 and TLR8 agonists Poly:IC and resiquimod could reverse the IL12 defect back to levels comparable to healthy donors. These findings resulted in a manuscript published in the Journal of Clinical Investigation and set the foundation of the next generation of dendritic cell vaccines.

2. Early pharmacodynamic effect of PD-1 blockade in human cancer.
   The PD-1 pathway plays a critical role restraining T cell function, including in exhausted CD8 T cells. My postdoctoral fellowship coincided with the introduction of PD-1 blockade in clinical oncology. In the laboratory of John Wherry, I defined the early pharmacodynamic effects of PD-1 blockade in human cancer patients, with early reinvigoration of CD8 T cells, as well as activation of CD4 and Tregs. In fact, Treg activation was a resistance mechanism of PD-1 blockade, and an early predictor of melanoma disease progression. The immunologic effect of PD-1 blockade in cancer patients occur early, peaking as early as day 7; by three weeks, the clinical response is largely determined. I also contributed to the understanding of the effect of PD-1 blockade on progenitor and terminal exhausted CD8 T cell subsets, and mechanisms of anti-PD-1 resistance, include exosomes.

d. **Huang AC** and Zappasodi R*. A decade of checkpoint blockade immunotherapy in melanoma: understanding the molecular basis for immune sensitivity and resistance. *Nature Immunology*, In Press

3. **Immunologic determinants of disease severity in COVID-19.**

In response to the COVID-19 pandemic, my lab has studied the impact of humoral dysfunction on immune protection against SARS-CoV-2 in cancer patients. In addition, I contributed to a number of COVID-19 related studies, including immune profiling of patients with severe COVID-19 and to a clinical trial of hydroxychloroquine for prophylaxis against COVID-19.


**Complete List of Published Work in My Bibliography:**

**D. RESEARCH SUPPORT**

**ACTIVE**

**Pew Stewart Scholars Award (Huang, A)** 7/1/2022 – 6/30/2026

W.W.Smith Charitable Trust

Molecular mechanisms of single-agent and combination checkpoint blockade.
The goal of this project is to determine the molecular mechanisms of aCTLA4, aPD-1, aCTLA4+aPD-1, and aCTLA4+aLag3 therapy.

Role: PI

**MRFBC Young Investigator Team Award (Huang, A)**
7/1/2022 – 6/30/2024
Melanoma Research Foundation
Exhausted CD8 T cells as an early indicator of lymph node metastasis and the need for adjuvant therapy in Stage II melanoma
The goal of this project is to determine whether early initiation of exhausted CD8 T cells is associated with anti-PD-1 response in Stage II melanoma.
Role: PI

**Damon Runyon Clinical Investigator Award and Doris Duke Clinical Scientist Development Award (Huang, A)**
7/1/2021 – 6/30/2024
Damon Runyon and Doris Duke
Shared antigen and neoantigen-specific T cells in checkpoint blockade efficacy and toxicity
The goal of this project is to understand how shared antigen and neoantigen-specific T cells have differentiation contributions to clinical efficacy and toxicity in checkpoint blockade.
*This award is being co-funded by the Doris Duke Charitable Foundation and Damon Runyon Research Foundation.*
Role: PI

**2021 ACC Pilot Grant Program Award / P30 CA016520 (Huang, A and Mitchell, T.)**
1/1/2021 – 11/30/2025
Abramson Cancer Center / NCI
Role of progenitor exhausted CD8 T cells in anti-PD-1 efficacy in human melanoma
The goal of this project is to understand the progenitor-exhausted CD8 T cells and their role in checkpoint blockade.
Role: PI

**2021 Skin Cancer Spore Career Enhancement Program Award (Huang, A)**
12/1/2021 – 12/31/2023
Abramson Cancer Center / NCI
Cellular and Molecular Mechanism of combination checkpoint blockade
The goal of this project is to understand cellular and transcriptional mechanism of the anti-PD-1 and anti-CTLA4 doublet therapy and anti-PD-1 and anti-Lag3 double therapy in human melanoma.
Role: PI

**K08CA230157 (Huang, A)**
7/1/2019 - 6/30/2024
NIH / NCI
Role of tumor burden in limiting durable reinvigoration by PD-1 blockade
The major goal of this project is to understand 1) whether high tumor burden results in quantitative and qualitative defect on tumor-specific T cells, and 2) decrease in tumor burden results in durable reinvigoration of exhausted CD8 T cells.
Role: PI

**R01CA273018 (Huang, A)**
9/1/22-8/31/27
NIH/NCI
Role of progenitor exhausted CD8 T cells and the progenitor niche in anti-PD-1 efficacy
The goal of this project is to understand the impact of anti-PD-1 on progenitor exhausted CD8 T cells and the importance of the progenitor niche in sustaining progenitor-exhausted CD8 T cells.
Role: PI
NAME: Li, Mingyao

eRA COMMONS USER NAME (credential, e.g., agency login): liming

POSITION TITLE: Professor of Biostatistics, Statistics, and Digital Pathology

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

<table>
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<tr>
<th>INSTITUTION AND LOCATION</th>
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<th>FIELD OF STUDY</th>
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<tbody>
<tr>
<td>Nankai University, Tianjin, China</td>
<td>B.S.</td>
<td>07/1996</td>
<td>Mathematics</td>
</tr>
<tr>
<td>Nankai University, Tianjin, China</td>
<td>M.S.</td>
<td>07/1999</td>
<td>Mathematics</td>
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<tr>
<td>University of Michigan, Ann Arbor, MI</td>
<td>M.S.</td>
<td>05/2002</td>
<td>Biostatistics</td>
</tr>
<tr>
<td>University of Michigan, Ann Arbor, MI</td>
<td>Ph.D.</td>
<td>05/2005</td>
<td>Biostatistics</td>
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</table>

A. Personal Statement

I am a statistical geneticist and biostatistician motivated in my research to identify genes that determine human variation and diseases. My research program involves two synergistic components: a methodological component focused on developing novel statistical methods for statistical genetics and genomics and an applied component focused on using these methods in clinical and biological studies. To develop my research program, I take a multidisciplinary approach that integrates methods drawn from statistics, machine learning, bioinformatics, and computational biology. I work closely with biomedical researchers to elucidate the structure and function of genomes and to translate genetics and genomics knowledge into human health. Since I started my faculty position at Penn in 2006, I have successfully led several NIH grants as a PI. I have substantial experience with the analysis of next-generation sequencing data, particularly in single-cell and spatial transcriptomics (ST). As shown below, I have published papers on single-cell RNA-seq and its applications in human diseases. Recently, I also published a spatial clustering and spatially variable gene detection method for ST data analysis in Nature Methods. More recently, I also expanded my expertise to computational pathology as processing and analysis of histology images are critical in ST data analysis. Given my expertise in computational pathology, I now have a secondary faculty appointment in the Department of Pathology and Laboratory Medicine at Penn. I have established close collaborations with the Pathology faculty, particularly those in anatomical and digital pathology.

I believe I have the expertise and leadership to serve as a co-PI to lead the proposed synergy project that aims to study the immunogenicity of early metastasis in sentinel lymph nodes by spatial transcriptomics analysis. This project will generate spatial transcriptomics data using the 10X Visium platform. Since Visium does not have a single-cell resolution, we will utilize iSTAR, a machine learning method that my lab recently developed to enhance gene expression resolution to near single-cell level. iSTAR leverages high-resolution spatial information provided by histology images and has shown promising performance in our preliminary data. We will apply iSTAR to the Visium data generated from normal and metastasized lymph nodes and use advanced computational methods to study immunological consequences of early metastasis and identify candidate predictive markers of recurrence in Stage III melanoma. This project is based upon my newly established collaboration with Dr. Alexandar Huang (human immunology) and Dr. Giorgors Karakousis (clinical melanoma). I am very excited about this collaboration and I hope to contribute my computational expertise to advance our understanding of the immunological consequences of early metastasis in melanoma.
I have not published research or research product under any other name.

Ongoing and recently completed projects that I would like to highlight include:

**R01GM125301**  
Li (PI)/Zhang (MPI)  
09/01/2017-08/31/2023  
Statistical methods for single-cell transcriptomics

**R01EY030192**  
Li (PI)  
05/01/2019 – 04/30/2023  
Single-cell transcriptomic analysis of human retina

**R21HL156234**  
Li (PI)  
07/15/2021-06/30/2023  
Integrative analysis of bulk and single-cell RNA-seq data for cardiometabolic disease

Below are papers that I would like to highlight to demonstrate my expertise in single-cell and spatial transcriptomics:


**B. Positions, Scientific Appointments, and Honors**

2022 – Professor of Digital Pathology (secondary), Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine

2021 – Director, Statistical Center for Single-Cell and Spatial Genomics, University of Pennsylvania School of Medicine

2019 – Chair, Graduate Program in Biostatistics, Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine

2017 – Professor of Biostatistics, Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine; Professor of Statistics (secondary), Department of Statistics and Data Science, University of Pennsylvania Wharton School of Business

2016 – Director of Biostatistics, The Gene Therapy Program and Orphan Disease Center, University of Pennsylvania School of Medicine

2015 – 2017 Associate Professor (secondary), Department of Statistics, University of Pennsylvania Wharton School of Business

2014 – 2017 Associate Professor (secondary), Department of Computer and Information Science, University of Pennsylvania School of Engineering and Applied Sciences

2012 – 2017 Associate Professor of Biostatistics (with tenure), Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine

2006 – Senior Scholar, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine

2006 – Faculty Member, Genomics and Computational Biology Graduate Program, University of
Pennsylvania School of Medicine
2006 – 2012 Assistant Professor of Biostatistics, Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine
2005 Postdoctoral Research Fellow, Center for Statistical Genetics, University of Michigan

Other Experience and Professional Memberships
2022 – Brown University COBRE Center for Computational Biology of Human Disease, Advisor Board
2021 – PLOS Computational Biology, Associate Editor
2021 – PLOS Genetics, Associate Editor
2021 – NIH Director’s Early Independence Awards (DP5), Reviewer
2020 – Annals of Applied Statistics, Associate Editor
2020 – Human Genetics and Genomics Advances, Associate Editor
2020 – NIH Special Emphasis Panel on Fellowships in Cell Biology, Developmental Biology and Bioengineering, Reviewer
2020 – NIGMS Early Stage Investigator R35/MIRA35 Review Panel, NIH, Reviewer
2019 – VA Office of Research and Development, Mental Health Study Section, Reviewer
2018 – NIH Ocular Surface, Cornea, and Refractive Error special emphasis panel within the Brain Disorders and Clinical Neuroscience IRG, Reviewer
2013, 2017 – NIH Genes Genomes and Genetics Special Emphasis Panel, Reviewer
2015 – Statistics in Biosciences, Associate Editor
2014 – 2019 – List of Experts, Shriners Hospitals for Children
2014 – 2017 – University of North Carolina at Chapel Hill OPPERA Study, Advisory Board
2013 – 2017 – Center for Inherited Disease Research Access Committee of NHGRI, Regular Member
2013 – American Association for the Advancement of Science, Member
2012 – NIGMS Special Emphasis Panel, NIH, Reviewer
2011 – 2017 – Genomics, Computational Biology and Technology Study Section, NIH, Regular Member
2010 – Frontiers in Statistical Genetics and Methodology, Associate Editor
2010 – Behavior Genetics and Epidemiology Study Section, NIH, Reviewer
2009, 2019 – Genomics, Computational Biology and Technology Study Section, NIH, Reviewer
2009, 2010 – NIDDK Special Emphasis Panel, Reviewer
2009 – 2017 – Briefings in Bioinformatics, Editorial Board Member
2003 – Eastern North American Region of the International Biometric Society, Member
2002 – American Statistical Association, Member
2001 – American Society of Human Genetics, Member

Honors
2022 Research Excellence Award, American Statistical Association Philadelphia Chapter
2021 Fellow, American Association for the Advancement of Science (AAAS)
2018 Fellow, American Statistical Association
2014 Elected Member, International Statistical Institute
2006 McCabe Pilot Award, University of Pennsylvania School of Medicine
2005 ENAR Distinguished Student Paper Award, Eastern North American Region of the Biometric Society
2005 C.W. Cotterman Best Student Paper Award, American Journal of Human Genetics
2004 – 2005 Rackham Predoctoral Fellowship, University of Michigan Rackham Graduate School

C. Contributions to Science

1. I was originally trained as a statistical geneticist at the University of Michigan by Drs. Michael Boehnke and Goncalo Abecasis. In the early stage of my career, my research was focused on developing statistical methods to identify genetic variations that contribute to disease risk. I have developed a number of statistical methods and tools for gene mapping of complex diseases. These include methods for joint modeling of linkage and association, analysis of quantitative traits, analysis of rare variants and pathways, methods for population stratification correction, methods for copy number variation detection, and methods for analysis of admixed populations. These methods integrate linkage and linkage disequilibrium information together with prior biological knowledge in elucidating disease causal variants for complex human diseases.


2. In addition to developing statistical methods to analyze genetic variations at the DNA level, I have also developed several statistical methods and computational tools to analyze data generated from RNA-seq studies. These include methods for isoform-specific gene expression estimation, differential expression, differential splicing, splicing QTL, and allele-specific gene expression analyses. Since 2016, I have expanded my research into single-cell genomics and have developed a series of methods for single-cell data analysis. The methods that I developed cover a wide range of topics, including gene expression denoising, clustering with batch effect removal, transcriptional bursting, alternative splicing, single-cell multi-omics data integration, and circadian phase inference.


3. I have also developed a series of methods that aim to integrate single-cell RNA-seq and bulk RNA-seq data for cell type deconvolution analysis. The MuSiC method that we developed is among one of the most popular cell type deconvolution algorithms in the field.


4. Since 2020, I started to work on problems related to spatial transcriptomics data analysis. Spatial transcriptomics enables gene expression profiling with spatial information in tissues and represents the latest frontier in genomics. I have developed methods for spatial clustering and spatially variable gene detection. In addition, I am working on several other computational problems for spatial
transcriptomics data analysis. I am particularly interested in developing methods that can integrate gene expression and histology images and maximally extract information in spatial transcriptomics.


c. Hu J, Coleman K, Lee EB, Kadara H, Wang L, Li M: Deciphering tumor ecosystems at super-resolution from spatial transcriptomics with TESLA. bioRxiv; DOI: [https://doi.org/10.1101/2022.11.05.515256](https://doi.org/10.1101/2022.11.05.515256)

5. **Other than statistical methods development, I have also made significant contributions to gene mapping studies of cardiometabolic disease and age-related macular degeneration.** I was the lead statistical geneticist on several large-scale gene mapping studies at Penn, including genetic studies of coronary artery disease, heart failure, high density lipoprotein cholesterol, and age-related macular degeneration. Such collaborations benefited my own methodological research in providing challenging statistical problems and also benefited my collaborators in providing them the most state-of-art tools and methods for analysis of their complex genetic and genomic data.


**Complete List of Published Work in MyBibliography:**
[http://www.ncbi.nlm.nih.gov/sites/myncbi/1rK6Ds5esp7Qg/bibliography/47220574/public/?sort=date&direction=ascending](http://www.ncbi.nlm.nih.gov/sites/myncbi/1rK6Ds5esp7Qg/bibliography/47220574/public/?sort=date&direction=ascending) (I have published >200 peer-reviewed papers with an H-index >75 and i10-index >130)
NAME: KARAKOUSIS, Giorgos C.

POSITION TITLE: Professor of Surgery, Hospital of the University of Pennsylvania

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

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>Completion Date MM/YYYY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yale University</td>
<td>B.S., M.S.</td>
<td>05/1996</td>
<td>Molecular Bioph and Biochem Medicine</td>
</tr>
<tr>
<td>University of Pennsylvania School of Medicine</td>
<td>M.D.</td>
<td>05/2001</td>
<td>Medicine</td>
</tr>
</tbody>
</table>

A. PERSONAL STATEMENT

I am a board certified surgical oncologist, and Chair of the Cancer Committee of the Abramson Cancer Center. Clinically, I treat hundreds of patients every year with skin cancers at various stages. I am on the Melanoma Leadership team in the Cancer Service Line at PennMedicine, and represent Penn Melanoma at the National Comprehensive Cancer Network (NCCN) meetings. I have recently served as the Chair of the Melanoma Disease Site Working Group for the Society of Surgical Oncology, active member of the ECOG-ACRIN melanoma committee, and am on the editorial board melanoma section for two journals. I am chair of the Scientific Committee of the Sentinel Lymph Node Working Group. At UPenn, I am the principal investigator of IRB approved study protocol for tissue collection of melanoma and other skin cancers which is critical for Core B. I have been heavily involved in establishing the melanoma SPORE tissue collection infrastructure as outlined in Core A and B that has enabled the collection and distribution to laboratories at UPENN and Wistar of > 11,000 clinically annotated biospecimens over the past 5 years. Scientifically, I have over 300 peer-reviewed publications. I have played an instrumental role in developing new biomarkers of response to immune checkpoint inhibition in Stage IV and Stage III melanoma, and in the development of mitotic index as a biomarker of poor prognosis in thin melanoma. I have also conducted extensive epidemiological and mechanistic preclinical studies with SPORE collaborators to better characterize the microenvironment of the sentinel lymph node, and how to utilize the sentinel lymph node biopsy information to improve clinical care in the changing landscape of early stage melanoma therapy. I currently serve as Project co-Leader for SPORE Project 3, and co-Investigator on Core B of our Penn-Wistar SPORE in melanoma.

B. POSITIONS AND HONORS

Positions and Employment

<table>
<thead>
<tr>
<th>Years</th>
<th>Position</th>
<th>Institution and Location</th>
</tr>
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<tbody>
<tr>
<td>2001-2002</td>
<td>Intern, General Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
</tr>
<tr>
<td>2002-2004</td>
<td>Resident, General Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
</tr>
<tr>
<td>2004-2006</td>
<td>Research Fellow, Department of Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
</tr>
<tr>
<td>2006-2008</td>
<td>Resident, General Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
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<tr>
<td>2008-2010</td>
<td>Fellow in Surgical Oncology</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
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<tr>
<td>2010-2017</td>
<td>Assistant Professor of Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
</tr>
<tr>
<td>2017-present</td>
<td>Associate Professor of Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
</tr>
<tr>
<td>2022-present</td>
<td>Professor of Surgery</td>
<td>Hospital of the U. of Pennsylvania</td>
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<tr>
<td>2014-present</td>
<td>Chair, Cancer Committee</td>
<td>Abramson Cancer Center, Perelman School of Medicine</td>
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<tr>
<td>2015-present</td>
<td>Member, Melanoma/Sarcoma Disease Site Working Group</td>
<td></td>
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<tr>
<td>2017-2018</td>
<td>Vice-Chair Melanoma/Sarcoma Disease Site Working Group</td>
<td>Society of Surgical Oncology</td>
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<tr>
<td>2018-2020</td>
<td>Chair, Melanoma Disease Site Working Group</td>
<td>Society of Surgical Oncology</td>
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<tr>
<td>2019-present</td>
<td>National Comprehensive Cancer Network Panel member (Melanoma)</td>
<td></td>
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<tr>
<td>2015-present</td>
<td>Executive Committee, Sentinel Lymph Node Working Group</td>
<td>Chair of the Scientific Committee (2021-present)</td>
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Honors and Awards
Magna Cum Laude (Yale University, New Haven, CT); Phi Beta Kappa (Yale University, New Haven, CT), Hellenic Medical Society of New York Award, 1999 (New York, NY); William T. Inouye Resident Teaching Award, 2006 (Department of Surgery, University of Pennsylvania, Philadelphia, PA); Traveling Fellowship to the Surgery Branch of National Cancer Institute, Bethesda, MD, 2009 (Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY); Doctor of the Year Award, Melanoma International Foundation (2016); Castle Connolly Top Doctor Philadelphia (2017-19),Philadelphia Magazine’s Top Doctor (2018-21); Humanitarian Award, Melanoma Research Foundation (Philadelphia gala, 2019); Penn Department of Surgery Mentorship Award (2020); James IV Association of Surgeons Travelling fellowship (2021); Penn Department of Surgery Jon B. Morris Faculty Teaching Award (2021); Department of Surgery Undergraduate Medical Education (UME) Teaching Award of Distinction (2021); Penn Pearls Teaching Award (2022); Department of Surgery Undergraduate Medical Education (UME) Teaching Award of Distinction (2022)

C. CONTRIBUTION TO SCIENCE
1. Through my clinical work and participation in clinical trials, I have collaborated in translational projects with immunotherapists and basic scientists to better understand and predict immune responses to immune checkpoint blockade therapy in patients with locally advanced or metastatic melanoma.

2. My other research work has focused on identifying prognostic biomarkers in patients with patients with melanoma. Some of the earlier work that I have been involved in helped to characterize the important prognostic significance of the mitotic index in patients with thin (≤1 mm) melanoma. Mitotic index was incorporated into the 7th edition American Joint Commission on Cancer (AJCC) staging system for melanoma for T1 lesions; moreover, it was recognized as a potentially important prognostic factor for determining suitability for SLN biopsy in select patients with thin melanoma.

3. Additional studies I have been involved with further characterized other prognostic factors in melanoma and melanocytic lesions, including the local immune response as measured by tumor infiltrating lymphocytes and regression, microsatellites, Clark level in patients with thin melanoma, age, lymphovascular invasion and lymph node characteristics.


Complete List of Published Work in My Bibliography:  

### D. RESEARCH SUPPORT

**Ongoing Research Support**

Melanoma SPORE grant Project 3 5P50CA261608-02 (PIs: Amaravadi, Herlyn)

Immunotherapy strategies for early-stage melanoma  
Role: Clinical Co-Leader PROJECT 3

**Previous Research Support**

<table>
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<tr>
<th>Grant Number</th>
<th>Start Date</th>
<th>End Date</th>
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<td>09/2017</td>
<td>07/31/2021</td>
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<tr>
<td>NIH/NCI</td>
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<tr>
<td>PI: Kao/Dorsey Circulating Tumor Analyses And Molecular Profiling</td>
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<tr>
<td>Melanoma Spore Pilot Grant*</td>
<td>10/2018-08/30/2020</td>
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<tr>
<td>Control Tissue Study for the Neoadjuvant PD-1 Blockade in Patients With Stage IIB/C Clinical Melanoma</td>
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<tr>
<td>PI: Xu, X</td>
<td>Role: Co-PI</td>
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<td>*From NIH Melanoma SPORE Grant P50-CA174523 (PI: Herlyn)</td>
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<td>Melanoma Spore Pilot Grant</td>
<td>09/2016-09/2018</td>
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<td>Age-related degradation of lymphatic vessels and increased angiogenesis may contribute to a higher incidence of visceral metastases in aged patients</td>
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<td>PI: Weeraratna, A</td>
<td>Role: Co-investigator</td>
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Development of new antibody bioconjugated nanoparticle based-lymphatic dyes for identification of melanoma micrometastases in sentinel lymph nodes using multispectral photoacoustic imaging
PI: Karakousis, G

P01 CA 114046 12/2011-08/2013
Targeted Therapies in Melanoma
NIH/NCI
PI: M. Herlyn
Role: Core Co-Director, Core B: Pathology Core
**Budget Justification**

**Personnel: ($25,000)**

**Dr. Alexander Huang, Co-PI, 0 mon cal:** Dr. Huang will oversee the 10x Visium spatial transcriptomic studies. Dr. Huang, in collaboration with Dr. Li and Dr. Karakousis will oversee major experimental design, data analyses, interpretation of the data, and preparation of the manuscripts.

**Dr. Mingyao Li, Co-PI, 0 mon cal:** Dr. Li is an expert in statistical genetics and genomics. She will oversee the spatial transcriptomics data analysis, and will meet with the research team on a weekly basis to discuss results and preparation of manuscripts.

**Dr. Giorgos Karakousis Co-PI, 0 mon cal.** Dr. Karakousis will oversee the acquisition of SLN+ and SLN- lymph nodes, and clinical annotation, as well as the experimental design, interpretation of the data, and preparation of the manuscript.

**Tarek Azar, postdoc, ~1.6 mon cal.:** Tarek will carry out all spatial transcriptomic experiments, interpret and analyze the data, and prepare the manuscripts, under Dr. Huang’s supervision.

**Ms. Hanying Yan, Biostatistician, ~2.7 mon cal.:** Ms. Yan is a Biostatistician in Dr. Li’s lab. Ms. Yan will conduct the statistical and bioinformatics analysis of the spatial transcriptomics data under Dr. Li’s supervision.

**Supplies: ($80,000)**

**10x Visium ($40,000):** The proposed spatial transcriptomics require 10x Visium reagents, including whole transcriptome RNA probe, and gene expression slide kits for 20 samples.

**Core services, histology core and high throughput sequencing core: ($35,000):** The proposed studies will require 1. Sectioning, slide preparation, H&E staining through the Molecular Pathology and Imaging Core and 2. Sequencing through the high throughput sequencing core.
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<th>Direct Costs</th>
<th>Effort</th>
<th>CM</th>
<th>Salary</th>
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<tr>
<td>Alex Huang-PI</td>
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<td>0.00</td>
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<tr>
<td>Mingyao LI-Col</td>
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<td>0.00</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Giorgos Karakousis - Col</td>
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<td>Tarek Azar - Postdoc</td>
<td>14%</td>
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<td>Hanying Yang - Biostatstician</td>
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<td>$50,000</td>
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