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TITLE: Defining and Therapeutically Targeting the Immunological Landscape of SF3B1-Mutant Breast Cancer

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CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center, Seattle, WA

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14. ABSTRACT

The core RNA splicing factor SF3B1 is recurrently mutated in breast cancer. These mutations occur as focal missense changes that alter SF3B1's normal role in RNA splicing. As a consequence, cells bearing these recurrent changes exhibit changes in splicing affecting hundreds of genes. We found that these recurrent splicing changes in breast cancers bearing *SF3B1* mutations resulted in the widespread production of abnormal mRNAs that encode novel proteins, which could result in the generation of neoepitopes that are recognizable by the immune system. We are therefore testing the hypothesis that splicing errors in breast cancer give rise to novel antigens that may facilitate recognition of tumor cells by the immune system.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

SF3B1, which encodes a core RNA splicing factor, is recurrently mutated in breast cancer. *SF3B1* mutations induce widespread mis-splicing that results in production of aberrant mRNAs encoding hundreds of novel peptides. These potential neoantigens are shared across all *SF3B1*-mutant breast cancers. Here, we propose to test the hypothesis that breast cancers harboring recurrent somatic mutations in *SF3B1* are uniquely susceptible to immunotherapy. We will: (1) Determine how *SF3B1* mutations alter the immunopeptidome by triggering neoepitope production, and (2) Assess the immunological consequences of *SF3B1* mutations and their potential as immunotherapeutic targets in *SF3B1*-mutant breast cancer.

2. KEYWORDS:

Breast cancer, RNA splicing, SF3B1, immunotherapy

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Identify mis-spliced mRNAs encoding novel peptides in *SF3B1*-mutant breast cancer.

Milestone(s): (1) Create mouse models of *SF3B1*-mutant breast cancer, (2) Identify all mis-spliced mRNAs in both mouse (E0771 and 4T1 models) and human breast cancer with *SF3B1* mutations.
Timeline: Months 1-12. 50% complete.

Major Task 2: Determine the subset of mis-spliced mRNAs that are efficiently translated into proteins.

Milestone(s): (1) Identify mis-spliced mRNAs that are translated in breast cancer with *SF3B1* mutations.
Timeline: Months 6-24. 25% complete.

Major Task 3: Identify *SF3B1* mutation-dependent neoepitopes that are presented by MHC class I.

Milestone(s): (1) Create breast cancer cells expressing defined MHC class I alleles, (2) Identify peptides presented by MHC class I in *SF3B1*-mutant breast cancer cells.
Timeline: Months 6-36. 25% complete.

Major Task 4: Assess immunogenicity of wild-type versus *SF3B1*-mutant tumors in mice.

Milestone(s): (1) Tumor growth curve data in immune-competent versus -incompetent mice, (2) Identify influence of *SF3B1* mutations on tumor infiltrating cell composition (3) Determine effects of checkpoint blockade with or with CDK4/6 inhibition on mutant versus wild-type tumours.
Timeline: Months 6-36. 0% complete.

Major Task 5: Assess immunogenicity of wild-type versus *SF3B1*-mutant human breast tumors.

Milestone(s): (1) Identification of banked breast cancer tissue samples from 10-15 *SF3B1*-mutant and 10-15 wild-type tumors, (2) Immunohistochemistry staining and analysis of PD-L1 status and CD8 T cell infiltration for *SF3B1*-mutant vs. wild-type human tumor tissue samples.
Timeline: Months 6-24. 25% complete.

Major Task 6: Identify and profile T cells specific for *SF3B1* mutation-dependent neoepitopes.

Milestone(s): (1) Produce peptide-MHC tetramers loaded with *SF3B1* mutation-dependent epitopes, (2) Identify endogenous *SF3B1* mutation-dependent epitope-specific T cells (3) Functional validation of *SF3B1* mutation-dependent epitopes.
Timeline: Months 12-36. 0% complete.

What was accomplished under these goals?

Major Task 1: Identify mis-spliced mRNAs encoding novel peptides in *SF3B1*-mutant breast cancer.

Subtask 1. Create syngeneic mouse models of *SF3B1*-mutant and wild-type breast cancer. Knock in *SF3B1*K700E and *SF3B1*K700K into E0771 and 4T1 cells and confirm successful engraftment. (RKB, EWN)

We designed CRISPR/Cas9 sgRNAs to introduce the *SF3B1*K700E mutation in mouse breast cancer cells. Although we were able to use these sgRNAs to introduce the desired mutation in a polyclonal setting, we were unable to obtain monoclonal populations bearing the desired mutation. We therefore changed strategy and are now using a doxycycline-inducible expression system to express WT or mutant SF3B1. We used this strategy to generate models of mutant SF3B1 in human breast cancer cells (see **Figure 1**) and so are now using it in mouse breast cancer cells.

Subtask 2. Perform RNA-seq analysis of mouse tumors (E0771 and 4T1 models). Engraft *SF3B1*-mutant and WT cells, collect tumors, perform RNA-seq, and identify differentially spliced isoforms in *SF3B1*-mutant vs. WT tumors. (RKB)

We have not yet performed work for this subtask.

Subtask 3. Perform RNA-seq analysis of human tumors. Identify differentially spliced isoforms in *SF3B1*-mutant vs. WT breast adenocarcinomas in two cohorts (TCGA and FHCRC cohort). (RKB)

We analyzed RNA-seq data from these cohorts by quantifying isoform expression using two different methods: (1) the MISO algorithm, and (2) counting junction-spanning reads that distinguished between isoforms. We identified differential usage of cassette exons, competing 5' and 3' splice sites, mutually exclusive exons, and retained and constitutive introns to identify those that were differentially spliced in *SF3B1*-mutant and WT samples (see **Figure 2** for example such mis-spliced events).

Major Task 2: Determine the subset of mis-spliced mRNAs that are efficiently translated into proteins.

Subtask 1. Perform ribosome profiling on *SF3B1*-mutant and WT cultured mouse tumor cells (E0771 and 4T1) and human tumor cells (MCF-7 and T-47D; obtained from ATCC). (RKB)

We obtained WT and *SF3B1*K700E MCF-7 and T-47D cells, generated using CRISPR/Cas9 knockin, and used corresponding RNA sequencing data from these cells to quantify differential isoform expression. These analyses confirmed that these cell lines recapitulated the patterns of mis-splicing observed in human primary tumors (see **Figures 1-2**).

Subtask 2. Analyze ribosome profiling data to measure isoform-specific translation. Identify mis-spliced isoforms that are specifically expressed in SF3B1-mutant, but not WT, cells and also translated. (RKB)

We have not yet performed work for this subtask.

Major Task 3: Identify *SF3B1* mutation-dependent neoepitopes that are presented by MHC class I.

Subtask 1. Ectopically express defined alleles of MHC class I in mouse tumor cells (H-2Kd, H-2Ld or H-2Dd for 4T1; H-2Kb or H-2Db for E0771) and human tumor cells (HLA-A*0201 or HLA-A*2402 for MCF-7 and T-47D, obtained from ATCC). (RKB)

We cloned HLA alleles of interest into ectopic expression vectors and additionally cloned C-terminal His tags to allow for specific purification of the allele of interest. We confirmed successful overexpression via Western blot and cell-surface expression via flow cytometry.

Subtask 2. Identify bound epitopes. Purify MHC-peptide complexes from tumor cells expressing defined MHC alleles, elute peptides, and identify peptides with mass spectrometry and search against a custom epitope database. (RKB)

We have not yet performed work for this subtask.

Major Task 4: Assess immunogenicity of wild-type versus *SF3B1*-mutant tumors in mice.

Subtask 1. Compare tumor growth kinetics of *SF3B1*-mutant and wild-type tumors (E0771 and 4T1 models) in immunocompetent versus immune-incompetent (RAG-knockout) mice. (EWN)

In addition to obtaining all required approvals for mouse work, pilot experiments were performed using wild-type mice to measure tumor growth kinetics and optimize our cellular analysis strategies (see **Figure 3** for an example of the mass cytometry data collected as part of this optimization process).

Subtask 2. Compare immune cell composition in *SF3B1*-mutant versus wild-type tumors (E0771 and 4T1 models) in immunocompetent mice using mass cytometry and, in some cases, flow cytometry. This subtask will be performed separately from subtask 1 and therefore require 40 additional mice. (EWN)

We have not yet performed work for this task.

Subtask 3. Compare efficacy of checkpoint blockade immunotherapy for *SF3B1*-mutant versus wild-type tumors (E0771 and 4T1 models) with or without CDK4/6 inhibition. (EWN)

We have not yet performed work for this task.

Major Task 5: Assess immunogenicity of wild-type versus *SF3B1*-mutant human breast tumors.

Subtask 1. Screen breast cancer tissue repository samples (obtained from FHCRC repository) to identify *SF3B1*-mutant tumors and comparable wild-type controls. (RKB, EWN)

We have worked with the breast cancer tissue repository to genotype samples for *SF3B1* mutations. We have identified 4 samples with *SF3B1* mutations and 140 WT samples. We also identified an additional ~100 samples for which extracted DNA is already available (~100 samples), which will facilitate genotyping, as well as the subset for which DNA extraction is required prior to genotyping (~900 sample).

Subtask 2. Perform immunohistochemistry staining of CD8 and PD-L1 on slides from samples identified in subtask 1. (EWN)

We have not yet performed work for this task.

Major Task 6: Identify and profile T cells specific for *SF3B1* mutation-dependent neoepitopes.

Subtask 1. Synthesize peptides identified in Aim 1 and use these to make peptide-MHC tetramers for the appropriate mouse alleles. (EWN)

We have not yet performed work for this task.

Subtask 2. Use multiplex tetramer staining to screen for and profile *SF3B1* mutation-dependent epitope-specific T cells in mouse tumors (E0771 and 4T1 models). (EWN)

In addition to obtaining all required approvals for animal work, pilot experiments and training of personnel were performed to test and optimize the mass cytometry peptide-MHC tetramer staining approach (See **Figure 4** for an example test of peptide-MHC tetramer staining).

Subtask 3. Validate T cell epitopes in vitro with tumor reactivity assays and in vivo by vaccinating mice prior to tumor implantation (E0771 and 4T1 models). (EWN)

We have not yet performed work for this task.

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

Dr. Newell and other lab members worked with Anthony Cessna to develop his skills in mouse handling, tumor cell tissue culturing, flow cytometry, mass cytometry and general understanding of cellular immunology.

Under the Partnering PI Award (W81XWH-20-1-0596), Dr. Bradley worked one-on-one with Dr. Nicholas to mentor her as she learned skills for genomic data analysis.

How were the results disseminated to communities of interest?

Nothing to report.

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

For Major Task 1, we will introduce transgenic WT and mutant SF3B1 into E0771 and 4T1 cells, engraft into mice, and use RNA-seq to identify differentially spliced isoforms.

For Major Task 2, we will identify mis-spliced isoforms that are specifically expressed and translated in *SF3B1*-mutant cells.

For Major Task 3, we will optimize MHC purification protocols for *SF3B1*-mutant and WT cells expressing defined MHC alleles.

For Major Task 4, we will quantify tumor growth in immune-competent and immune-deficient mice.

For Major Task 5, we will optimize protocols for IHC analyses of immune infiltrates and continue identifying relevant tumor samples.

For Major Task 6, we will test and optimize peptide-MHC assays using candidate neoepitopes promoted by *SF3B1* mutations.

4. IMPACT:

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

Nothing to report.

What was the impact on other disciplines?

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

We had an extended slowdown in laboratory work at our institution due to COVID-19. Because of this slowdown, progress on Major Task 1 and Major Task 4 was slower than originally anticipated.

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Although we encountered an unexpected challenge with creating knockin cell lines for Major Task 1, we developed a good strategy (see accomplishments above - Major Task 1, Subtask 1) to overcome these difficulties and are now proceeding with their creation.

COVID-19 has delayed progress on the objectives of this project. In compliance with institutional policies and the Washington State "Stay Home" order announced March 23, 2020 by Governor Jay Inslee, lab operations were halted or slowed considerably from April 2020 through June 2021. As such there has been less wet lab effort, resulting in a delay of progress, especially on Major Tasks 1 and 4. However, the wet lab work continued as possible in accordance with federal, state and institutional policy, and in the coming project year, we anticipate being able to make up the time lost and do not expect a long-term impact to the project timeline.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Evan Newell*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*ORCID: 0000-0002-2889-243X*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Newell has helped in obtaining mouse work approvals and supervised cellular analysis.*
Funding Support:	*W81XWH-20-1-0597*

Name:	*Anthony Cessna*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-6983-560X*
Nearest person month worked:	*9*
Contribution to Project:	*Anthony Cessna has performed cell culture, mouse tumor inoculation, mass cytometry and tetramer staining optimization.*
Funding Support:	*W81XWH-20-1-0597*

Name:	*Shamin Li, PhD*
Project Role:	*Postdoctoral Researcher*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7669-7391*
Nearest person month worked:	*2*
Contribution to Project:	*Prior to her departure, Dr. Li assisted in the proper set up and execution of the mouse experiments, mass cytometry, and tetramer staining.*
Funding Support:	*W81XWH-20-1-0597*

Name:	*Amy Codd, PhD*
Project Role:	*Postdoctoral Researcher*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-4077-4743*
Nearest person month worked:	*1*
Contribution to Project:	*Following Dr. Li's departure, Dr. Codd provided support and oversight to Anthony Cessna in the execution of the mouse experiments, mass cytometry, and tetramer staining.*
Funding Support:	*W81XWH-20-1-0597*

Name:	*Robert Bradley*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*ORCID: 0000-0002-8046-1063*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Bradley has performed genomic data analysis and supervised the project.*
Funding Support:	*W81XWH-20-1-0596 (Partnering PI Award)*

Name:	*Taylor Nicholas*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*10*
Contribution to Project:	*Dr. Nicholas has performed genomic data analysis and cell culture experiments.*
Funding Support:	*W81XWH-20-1-0596 (Partnering PI Award)*
Name:	*Jamie Guenthoer*
Project Role:	*Staff Scientist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Guenthoer has worked with specimen identification and analysis.*
Funding Support:	*W81XWH-20-1-0596 (Partnering PI Award)*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Evan Newell, PhD

PREVIOUS AWARDS

**Newly Ended*

TDS IRC Award (Bleakley / Newell / Bradley)* 07/01/2020 – 06/30/2021 0.12 calendar months
Fred Hutchinson Cancer Research Institute
Next Generation Assays and Analysis of Graft-versus-Leukemia
The purpose of this project is to determine which technologies will best enable identification and tracking of leukemia-specific TCR in HCT donor cells and in HCT recipients and refine the TCR analytic methods that will facilitate understanding of leukemia antigen-TCR relationships.
Aim 1: Determine which technology or combination of technologies will best enable identification and tracking of leukemia-specific TCR in HCT donor cells and in HCT recipients.
Aim 2: Determine refinements in TCR analytic methods will facilitate understanding of leukemia antigen-TCR relationships.
Role: Co-PI

AI128914 (Stuart, K.)* 06/01/2020 – 05/31/2021 0.36 calendar months
NIH/NIAID (Subaward Only)
Assessing split-pool barcoding-based sequencing analysis (SPLiT-seq) of cellular protein and TCR sequence for high-throughput single-cell profiling of vaccine-specific T cell
The purpose of this project will be to adapt SPLiT-seq for high throughput parallel analysis of TCR (BCR also possible) and cellular profiles using DNA-tagged antibodies (a.k.a., CITEseq10, REAP-seq11, Ab-seq12).
Aim 1: Establish SPLiT-seq for the simultaneous evaluation of single-cell TCR sequence and protein expression profile
Aim 2: Perform a benchmark comparison of SPLiTseq with commercial approaches and adaptationRole: Subaward PI

Federal Agency Contact:
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U19 AI128914 (Stuart, K.)* 06/01/2019 – 05/31/2020 0.24 calendar months
NIH/NIAID
High dimensional profiling of naive B cell heterogeneity to identify novel relationships with the generation of broadly neutralizing antibodies
The goal of this project is to develop and optimize methods to better describe naïve B cell phenotypic diversity and to test the hypothesis that naïve bnAb precursor B cells are phenotypically restricted in HIV-naïve healthy donors.
Aim 1: Unbiased phenotypic profiling of human B cells to describe the range of phenotypic diversity within nonclass-switched B cells.
Aim 2: Identifying and profiling Env-specific B cells from HIV-naïve healthy donors using mass cytometry and single-cell sequencing.
Role: Subaward PI

Federal Agency Contact:
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IIRC Research Award (Newell)* 03/01/2019-02/28/2021 0.60 calendar months
Fred Hutchinson Cancer Research Center
Unravelling the role of MAIT cells in colorectal cancer (CRC) as an interface player between microbiome and cancer
The aim of this project is to fully profile MAIT cells at both the protein and the gene level primarily in colorectal cancer (but also non-small cell lung cancer and oral cancer) to decipher how these characteristics could be connected to gut microbiome and associated gene-level metagenomics profiles. This project will provide information for fundamental questions about the roles of MAIT cells and the associations between the microbiome and cancer risk, paving the way for therapeutic microbiome manipulations.
Aim 1: To assess relationships between MAIT cell phenotypic and functional heterogeneity and the overall immunological characteristics of the colorectal and lung tumor microenvironment using mass cytometry.
Aim 2: To explore programs associated with MAIT cell differentiation that occur in the context of the colorectal tumor microenvironment using the BD Rhapsody-AbSeq platform in conjunction with in vitro functional assays.
Aim 3. To Determine the extent to which gut microbes are critical determinants of MAIT cell phenotypes in colorectal cancer.
Role: PI

P30 CA015704 (Lynch)* 7/01/2019 – 6/30/2021 0.60 calendar months
NIH/NCI (New Investigator Award Only)
New Investigator Award (PI: Kim): Activating tumor-associated macrophages through blockade of interleukin-10
The proposal will study and define the functional state of tumor-associated macrophages (TAM) in human colorectal cancer liver metastasis, and the influence of interleukin-10 blockade on shifting TAMs from an immunosuppressive to a stimulatory phenotype using CyTOF and intravital imaging of tumor slice cultures. In this manner we hope to dissect the determinants of immune cell fate and guide rational immunotherapy design.
Aim 1. Test the hypothesis that human CRCLM macrophages exist in a dynamic but predominantly inhibitory state.
Aim 2. Test the hypothesis that IL10 blockade polarizes TAMs toward a stimulatory state.
Role: Co-Investigator

Federal Agency Contact:
Jacquelyn Saval
Grants Management Officer
National Cancer Institute
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VIDD Faculty Initiative (Newell) 07/01/2018 – 06/30/2019 0.60 Calendar Months
Fred Hutchinson Cancer Research Center
Assembling tools for profiling peripheral and tumor infiltrating EBV-specific T cell responses in nasopharyngeal carcinoma
The purpose of this project will be to assemble an effort to develop several generally applicable tools and begin

to apply them towards the better understanding the EBV-specific T cell response in nasopharyngeal carcinoma (NPC) cancer patients.
Aim 1: CyTOF assessment of peripheral blood derived EBV-specific T cells in NPC patients stratified by EBV viral load.
Aim 2: Evaluation of the relationships between EBV-specific T cell responses in blood vs. tumors of NPC patients.
Role: PI

The awards listed below were issued to Singapore Immunology Network prior to Dr. Newell's transfer to Fred Hutchinson Cancer Research Center. Award information is provided as available.

Singapore A*STAR, SIgN Core funding Newell (PI) 2012 – 2018
Study of antigen specific T cells. Role: PI

Singapore NMRC, TCR: Lim Seng Gee (PI) 2015 – 2018
Initiated studies investigating roles of antigen-specific T cells in HBV viral clearance.
Role Co-Investigator

NIH NIAID, R01 Robert Schreiber (PI) 2015 – 2018
Study of tumor neo-antigen specific T cells in mouse models of cancer.
Role: Subcontractor PI

NMRC, CS-IRG Choo Su Pin (PI) 2014 – 2018
Analysis of HBV and HCC specific T cell responses in the context of HCC radiotherapy and combination radiotherapy and immunotherapy.
Role: Co-Investigator

Singapore A*STAR, Singapore Immunology Network Clinical Immunomonitoring Platform
Laurent Renia (PI) 2013 – 2016
Development of mass cytometry platform to attract industrial collaborations related to high dimensional immune monitoring.
Role: Co-Investigator

Singapore A*STAR, JCO Full Research Grant Evan Newell (PI) 2013 – 2016
Development of novel methods for tagging antibodies and peptide-MHC tetramer with heavy metals to extend the utility of mass cytometry and foster industrial development of mass cytometry related technologies.
Role: Co-Investigator

Singapore NMRC, CS-IRG Lim Seng Gee (PI) 2014 – 2017
Mapping T cell responses to chronic HBV infection using highly multiplex peptide MHC tetramer staining as proposed here. Demonstrated ability to probe >500 different T cell antigen specificities simultaneously and developed novel methods for investigating relationships between various categories of HBV-specific T cell phenotypes, including exhaustion marker expression profiles.
Role: Co-Investigator

Singapore, NMRC, MOH IAF Cat. 2 platform Salvatore Albani (PI) 2015- March 2018
Developing MHC class II tetramer staining reagents using baculovirus insect expression to be shared with the platform group.
Role: Co-Investigator

CURRENT AWARDS

**Newly Awarded*

Fred Hutchinson Cancer Research Center (Newell) 07/01/2018 – 06/30/2023 No Dedicated Effort
New Development Funding
This support is provided by the Vaccine and Infectious Disease Division at the Fred Hutchinson Cancer Research Center, Seattle, WA, for the Principal Investigator to establish a laboratory within the division and is not restricted to any individual project.
Role: PI

Andy Hill Care Award (Newell) 08/01/2018 – 07/31/2023 No Dedicated Effort
Andy Hill Cancer Research Endowment
Distinguished Researcher Grant Award
The purpose of this award is to provide matching funds for the recruitment and retention of the Distinguished Researcher and is not restricted to any individual project.
Role: PI

Clinical Trial Agreement (Cheever)** 01/01/2019 – End of Study** 0.30 calendar months
Merck (– Newell Lab only)
CITN-13 (OTSP) A Phase II Trial of MK-3475 (pembrolizumab) and Interferon Gamma 1-b Combination Immunotherapy in Patients with Previously Treated Mycosis Fungoides and Sézary Syndrome (Treatment Group 1) and in Patients with Advanced Synovial Sarcoma (Treatment Group 2)
To assess the overall response rate (ORR) of MK-3475 (pembrolizumab) and IFN-gamma (Actimmune®) combination immunotherapy in subjects with previously treated Mycosis Fungoides or Sézary Syndrome**.**
Aim 1: Treatment Group 1: To assess the overall response rate (ORR) of MK-3475 (pembrolizumab) and IFN-gamma (Actimmune®) combination immunotherapy in subjects with previously treated Mycosis Fungoides or Sézary Syndrome.
Aim 2: Treatment Group 2: To determine whether the combination of interferon gamma-1b (ACTIMMUNE®) and MK-3475 (pembrolizumab) improves the ORR of pembrolizumab in patients with unresectable or metastatic synovial sarcoma.

****NOTE:*** Dr. Newell's role on this project extends from 06/01/2021 – 11/30/2021. During this period, his lab will perform Mass Cytometry work for the project.
Role: Co-Investigator

Merck Contracting Officer: Sloan Stribling
Title: Associate Director
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Mailstop RY34 A2109M
Phone: 732-594-2194
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HDC Accelerator Award (Gottardo, R.)* 11/01/2019 – 10/31/2021 (NCE) 0.30 calendar months
Fred Hutchinson Cancer Research Center (Newell Lab only)
Deep immune profiling of CAR T cells from pediatric and adult cohorts
The purpose of this project is to provide the first cross-institutional, cross-trial analysis of CD19 CAR-T products from pediatric and adult patients, generating a dataset large enough to enable biomarker discovery.

Resulting biomarkers related to CAR-T cell phenotypes and functions will provide new insights to improve response rates and decrease toxicity in future CAR-T cell products, directly benefiting patients.
Aim 1: To conduct exploratory studies to evaluate the phenotypes and functional aspects of pre-manufacturing T cells (PBMC, leukapheresis, selected CD4/CD8, as available) that correlate with clinical and laboratory outcomes in pediatric and adult recipients from two centers.
Aim 2: To conduct exploratory studies to evaluate the phenotypes and functional aspects of CD19 CAR-T cells from infused products that correlate with clinical and laboratory outcomes in infused recipients from two centers.
Aim 3: To evaluate differences in phenotype and function of pre-manufacturing T cells and infused CD19 CAR-T cells between pediatric and adult patients undergoing CAR-T cell therapy.
Role: Co-Investigator

Fred Hutch Contact: Katie Smolnycki
Email: ksmolnyc@fredhutch.org

P30 CA015704 (Lynch)* 04/01/2021 – 03/31/2022 0.45 calendar months
NIH/NCI
CCSG Pilot Award (PI: Newell): Longitudinal tracking of Merkel cell carcinoma specific T cell responses: testing biomarker utility of Merkel cell polyomavirus specific cells and associated cellular phenotypes
Our goal is to more deeply characterize MCPyV-specific cells, related populations of cells, and cancer unrelated antigen-specific cells and their clonalities over the course of anti-PD-1 treatment to gain insights.

Aim 1: Identification of novel biomarkers of immunotherapeutic response based on high dimensional T cell profiles.
Aim 2: Defining the identities and clonotypic relationships of T cell populations associated with immunotherapeutic response.

Federal Agency Contact:
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Research Agreement (Green/Gottardo) 07/1/2019 – 05/31/2022 0.60 calendar months
Allen Institute
Deep Immune Profiling of Patients with Multiple Myeloma
Aim 1: Evaluate the immune profile in blood and bone marrow in newly diagnosed patients with multiple myeloma before and after conventional induction therapy and autologous stem cell transplant in eligible patients.
Aim 2: To sequentially perturb the immune system of newly diagnosed patients with multiple myeloma after conventional induction therapy and autologous stem cell transplant (if eligible) in year 1 and year 2 of the longitudinal study with the seasonal influenza vaccine routinely offered to our clinic patients per calendar year and investigate the dynamic immune response to vaccination through return to homeostasis and relate these events to the healthy adult control group.
Role: Co-Investigator

Sponsor Contact: Ernie Coffey, MBA
Email: ecoffey@alleninstitute.org

U19 AI128914 (Stuart)** 07/19/2017 – 06/30/2022 1.20 calendar months
NIH/NIAID (Project 2 – Newell Lab only)
Immune Responses to Malaria and HIV Infection and Immunization: Project 2 (McElrath)
The main objective of this cooperative agreement is to support the identification of immune profiles that correlate with vaccine efficacy and are of potential relevance to protection against HIV-1 and P. falciparum infection.
Specific Aim 1: Comprehensively define molecular signatures of HIV-specific CD4+ and CD8+ T cells associated with a protective outcome against infection in vaccine recipients.
Specific Aim 2: Identify immune profiles and signatures with different adjuvant formulations that reliably predict and confer induction of immune potency and durability.
Specific Aim 3: Develop and apply a systematic platform to define the B cell immune repertoire associated with induction of anti-HIV broad neutralizing antibodies (bnAbs).
Role: Project 2 Co-Investigator

****NOTE:** Dr. Newell was added to this project in mid-year in Year 4 which began July 1, 2020.

Federal Agency Contact:
Michael W. Fato
Grants Management Officer
National Institute of Allergy and Infectious Diseases
BG 5601FL RM 4E50
5601 Fishers Ln

U19 AI128914 (Stuart)** 07/19/2017 – 06/30/2022 1.50 calendar months
NIH (subaward from SCRI) (total subaward costs)
Immune Responses to Malaria and HIV Infection and Immunization: Data Management & Analysis Core
The goal of this project is to develop a central data management system based on the LabKey Server to collect, store, manage, quality control (QC), analyze, and share data generated through our research projects, as well as develop software tools (in R) and processing pipelines to import, annotate, pre-process, and standardize data from common assay technologies for deposition into the data management system. The Core will also be responsible for establishing a central resource for study design, power calculations, and common statistical analyses to be undertaken within the different research projects.
Role: Co-investigator, Core PI
Specific Aim 1: Develop a central data management system for data collection, storage, and sharing.
Specific Aim 2: Develop tools to quality control, standardize, and annotate raw data to facilitate submission to ImmPort.
Specific Aim 3: Provide study design and statistical support.

****NOTE:** Dr. Newell replaced Dr. Gottardo as the Core PI on this project as of July 1, 2021 (Project Year 5).

Federal Agency Contact:
Michael W. Fato
Grants Management Officer
National Institute of Allergy and Infectious Diseases
BG 5601FL RM 4E50
5601 Fishers Ln

U19 AI128914 – Supp. (Stuart)** 07/01/2018 – 06/30/2022 0.90 calendar months
NIH (subaward from SCRI) (total subaward costs)
HIPC Database and Infrastructure Development

The goal of this project is to continue the development of ImmuneSpace. ImmuneSpace is a high-quality public web-interface to HIPC data, analysis tools and results. ImmuneSpace is one of the major deliverables of the HIPC program and will act as the central immunological hub, allowing experimentalists, statisticians, and bioinformaticians to freely retrieve, explore and compare data across studies.
Role: Co-investigator, Project PI

Specific Aim 1: Maintain and update the database and the overall infrastructure
Specific Aim 2: Develop new features to facilitate collaborative and integrative analyses and enable reproducible research
Specific Aim 3: Measure and improve user experience.

****NOTE:** Dr. Newell replaced Dr. Gottardo on this project as of July 1, 2021 (Project Year 5)

Federal Agency Contact:
Michael W. Fato
Grants Management Officer
National Institute of Allergy and Infectious Diseases
BG 5601FL RM 4E50
5601 Fishers Ln

(THIS AWARD)
BC191163P1 (Bradley, R.) 07/01/2020 – 06/30/2023 0.90 calendar months
Dept. of the Army -- USAMRAA
Defining and therapeutically targeting the immunological landscape of SF3B1-mutant breast cancer
This purpose of this project is to identify and analyze mis-spliced mRNAs encoding novel peptides in SF3B1-mutant breast cancer that efficiently translate into proteins; Identify SF3B1 mutation dependent neoepitopes that are presented by MHC class I; assess immunogenicity of wild-type versus SF3B1-mutant tumors (human and mice); and Identify and profile T cells specific for SF3B1 mutation-dependent neoepitopes.
Aim 1: Identify mis-spliced mRNAs encoding novel peptides in SF3B1-mutant breast cancer
Aim 2: Determine the subset of mis-spliced mRNAs that are efficiently translated into proteins
Aim 3: Identify SF3B1 mutation dependent neoepitopes that are presented by MHC class I
Aim 4: Assess immunogenicity of wild-type versus SF3B1-mutant tumors in mice
Aim 5: Assess immunogenicity of wild-type versus SF3B1-mutant human breast tumors
Role: Partnering PI

Federal Agency Contact:
Jamie A. Shortall
Grants Officer
Department of the Army
US Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014

R33 CA255893 (Seelig, G.)* 04/01/2021 – 03/31/2024 0.90 calendar months
NIH/NCI (Newell Lab Portion)
Joint receptor and protein expression immunophenotyping through split-pool barcoding
Single-cell immune repertoire sequencing can provide invaluable information about the response of the adaptive immune system to disease and therapy. However, existing approaches for pairing T-cell or B-cell receptor sequences at the single-cell level are still relatively low throughput and costly. These limitations are particularly acute for methods that aim to combine receptor sequences with complementary cell-type information, as

defined by cell-surface protein expression. Here, we propose to address these challenges and develop an affordable and high-throughput method for multi-modal single-cell immunophenotyping.
Role: Co-Investigator

Federal Agency Contact:
Dawn M. Mitchum
National Cancer Institute
BG 9609 MSC 9760
9609 Medical Center Drive
Bethesda, MD 20892-9760
Telephone: 240-276-5699
Email: Dawn.Mitchum@nih.gov

U54 CA242977-01 (Madeleine/Duerr) 7/1/2019 – 6/30/2024 0.24 calendar months
NIH/NCI
Cervical Cancer Prevention in Peru and the Dominican Republic
The aims of the proposed Clinical Trials Program span the continuum of cervical cancer prevention from vaccination through treatment. In Trial 1, we will identify new dosing schedules to improve immunogenicity of HPV vaccination among children living with HIV (CLWH). In Trial 2, we will ascertain the contribution of new technologies to refine algorithms for screening and triage of women living with HIV (WLWH). In Trial 3, we will assess non-surgical immune-based treatments of precancerous lesions to interrupt progression to cervical cancer in WLWH.

Trial 1: HPV immunoprevention by vaccination of unexposed children is not optimized for children living with HIV (CLWH). We will compare longer-term immune (anamnestic) responses among HIV-infected youth (ages 9-13 at the time of enrollment) after 1, 2, or 3 doses of 9-valent HPV vaccine.

- **Aim 1.** Characterize the B cell memory response (elicited by a booster dose of HPV vaccine) among CLWH who received 1, 2, or 3 doses of the 9-valent HPV vaccine.
- **Aim 2.** Determine the potency and breadth of neutralization of HPV monoclonal antibodies among CLWH described in Aim 1.
- **Aim 3.** Compare responses among CLWH receiving 1, 2, or 3 doses to HIV-uninfected children who receive the standard 2-dose regimen.

Trial 2: Cervical cancer screening and triage among WLWH needs to be refined to improve detection of precancerous lesions, reduce overtreatment, and facilitate implementation. We will develop a more efficient algorithm to detect high-grade cervical intraepithelial neoplasia (CIN2/3) by assessing standard of care (Pap test and visual inspection with acetic acid), HPV testing, and newer technologies including HPV E6/E7 expression and enhanced visual inspection (EVA) with automated visual evaluation (AVE).

- **Aim 1.** Optimize an algorithm that accurately and reproducibly predicts CIN2+ through the combination of assays for primary screening and triage of screen-positives.

Trial 3: Adjuvant and non-surgical strategies for treatment of CIN2/3 may decrease the need for primary or repeat surgical interventions in WLWH. Participants with CIN2/3 from Trial 2 will be offered to enroll in a 2x2 factorial randomized controlled trial of 9-valent HPV vaccine and sirolimus initiated before surgery.

- **Aim 1.** Evaluate whether 3 doses of vaccine decrease risk of CIN2/3 recurrence by 50%.

Role: Co-Investigator

Federal Agency Contact:
Sean Hine
National Cancer Institute (NCI)
Telephone: 240-276-6291

Email: hines@mail.nih.gov

R01 CA264646 (Newell)* 7/1/2021 – 06/30/2026 3.00 calendar months
NIH/NCI
The roles of EBV-specific T cells in response to checkpoint blockade immunotherapy of EBV-driven nasopharyngeal carcinoma
The purpose of this project is to improve understanding of T cell responses to nasopharyngeal carcinoma (NPC) by using complementary approaches to comparatively analyze the profiles of T cells in blood vs. tumor tissue that are specific for the EBV virus, other tumor associated antigens as well as bystander T cells specific for cancer unrelated antigens.
Specific Aim 1: Investigate the clinical relevance of NPC-associated CD8+ T cell phenotypes by assessing their transcriptional profiles, TCR clonal dynamics, and tumor reactivity in the NPC tumor and periphery.
Specific Aim 2: Characterize EBV-specific T cells response during combination anti-PD-1+anti-CTLA-4 immunotherapy treatment.
Specific Aim 3: Investigate T cell clonal dynamics associated with treatment-induced changes to the NPC-specific immune response.

Federal Agency Contact:
Jacquelyn Saval
Grants Management Specialist
NATIONAL CANCER INSTITUTE
savalj@mail.nih.gov
(240) 276-6312

UM1 AI068618 (McElrath, J.)* 12/01/2020 – 11/30/2027 1.20 calendar months
NIH/NIAID
HIV Vaccine Trials Network Laboratory Center
The overall goal of the HIV Vaccine Trials Network (HVTN) Laboratory Center (LC) is to conduct high quality, state-of-the-art laboratory-based research on behalf of the HVTN that will accelerate the development of a safe and efficacious HIV vaccine.
Specific Aim 1: Define and compare the profile and kinetics of innate and adaptive immunity induced by candidate HIV vaccine regimens in HVTN phase 1-2a trials, including the conduct of pre-specified go/no go studies for product advancement in all age groups.
Specific Aim 2: Develop new and improved methods to apply to the comprehensive assessment of potentially relevant, protective immune responses in pediatric and adult populations within experimental clinical research and clinical vaccine trials.
Specific Aim 3: Determine HIV vaccine efficacy in phase 2b-3 clinical trials, and identify correlates of protection against HIV infection, including host immunity, host genetics and viral genetics.
Specific Aim 4: Assess non-vaccine HIV prevention strategies, including immune prophylaxis with antibodies, alone or in combination with vaccines, in collaborative phase 1-3 efficacy trials, and oversee the conduct of laboratory studies to identify correlates of protection against HIV infection.
Specific Aim 5: Identify mucosal immune responses following HIV immune prophylaxis and vaccination that may be relevant to preventing or containing infection at the portal of entry.
Specific Aim 6: Provide the leadership and laboratory capacity to assess host immune responses to candidate vaccines for *M. tuberculosis* (Mtb) and other infections in all age groups.
Role: Co-Investigator

Federal Agency Contact:
Jason Lundgren
Grants Management Specialist
DHHS, NIH, NIAID, GMP

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Bethesda, MD 20892
(240) 669-2973
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OVERLAP

None.

OTHER – INTERNATIONAL COLLABORATIONS - PAST TWO YEARS (older collaborations and contact information available on request)

Presenter, 2019 CSCO Annual Conference 09/18-22/2019 0.0 calendar months
Chinese Society of Clinical Oncology
Xiamen, China
Funds involved: Reimbursed Travel Expenses; Honorarium

The major goal of this activity was to present on the lab's published and ongoing non-confidential work studying human T cell responses in the context of health, cancer and infectious diseases.

Presenter, CIRNO Symposium 10/29-31/2019 0.0 calendar months
Sungkyunkwan University
Seoul, South Korea
Funds involved: Reimbursed Travel Expenses; Honorarium

The major goal of this activity was to present the lab's published ongoing non-confidential work studying human T cell responses in the context of health, cancer and infectious diseases.

Advisory Role Ongoing 0.0 calendar months
immunoSCAPE PTE. LTD.
Singapore
Funds involved: Reimbursed Travel Expenses

Dr. Newell has an advisory role at immunoSCAPE PTE. LTD. Activities involve providing expertise on human immune profiling.

OTHER INTERNATIONAL RESOURCES PROVIDED (Non-monetary)

Dr. Newell has received de-identified human subjects samples under material transfer agreements from the following international collaborators: Dr. Bruce Robinson (University of Western Australia), Dr. Darren Lim (National Cancer Centre Singapore), Dr. Amit Jain (National Cancer Centre Singapore).

PREVIOUS, CURRENT, AND PENDING RESEARCH SUPPORT

BRADLEY, ROBERT K.

PREVIOUS SUPPORT

Title	Therapeutic targeting of spliceosomal---mutant acquired bone marrow failure disorders (W81XWH-16-1-0059-01)
Effort	0.24 CPM
Agency	Department of Defense
Grants Officer	Christin Helman Email: christine.e.helman.civ@mail.mil Phone: (301) 619-2265
Period	05/01/16 – 04/30/19
Funding	(total costs for year 3)
Goals	Dr. Bradley will be primarily responsible for the genomics studies in Aim 1, including SILAC experiments and computational data analysis, and CRISPR/Cas9 screens in Aim 2. Dr. Bradley will also contribute to compound library screening and subsequent hit validation in Aim 3. All studies conducted by Dr. Bradley will involve cell lines or computational analysis of data from cell lines, murine models, or primary patient samples.
Specific Aims	Aim 1. Identify unifying molecular abnormalities across spliceosomal mutations using combined genomic and proteomic approaches. Aim 2. Identify additional genes that are required for the survival of cells carrying different spliceosomal mutations with synthetic lethality screens. Aim 3. Identify novel therapeutic strategies specifically targeting mutant spliceosomal protein function.
Role	Co-Principal Investigator
Overlap	None

Title	Inhibition of RNA quality control in differentiation and aging cells
Effort	0.60 CPM
Agency	Ellison Medical Foundation
Grants Officer	Kevin Lee Email: klee@ellisonfoundation.org Phone: (212) 577-9255
Period	09/01/13 – 08/31/17
Funding	(total costs for year 4)
Goals	This application proposes to use the developing and aging heart as a tractable and biomedically relevant system to determine the mechanistic origins and global consequences of RNA surveillance inhibition. The proposed research will provide the first characterization of a novel cellular dysfunction that may contribute to aging-associated disorders ranging from age-related macular degeneration to neurodegenerative diseases.
Specific Aims	Aim 1. Identify RNAs degraded by NMD that are stabilized during *in vitro* cardiac differentiation. Aim 2. Determine which of these RNAs are present and translated *in vivo* in aged mouse heart. Aim 3. Determine the mechanistic origins of developmental and age-associated NMD inhibition.
Role	Principal Investigator
Overlap	None

Title	Biological mechanisms and therapeutic opportunities for MDS with spliceosomal mutations
Effort	0.60 CPM
Agency	EvansMDS Foundation
Grants Officer	Michael Lewis Email: mdl@epefoundation.org Phone: (978) 494-6009
Period	09/01/15 – 08/31/17

Funding	(total costs for year 2)
Goals	We propose to use a combination of experimental and computational approaches to determine how mutations that affect RNA splicing give rise to MDS. We will use this knowledge to conduct drug discovery experiments, wherein we will screen thousands of molecules to identify compounds that specifically target malignant cells carrying these mutations. The long-term goal of this research is to lay the foundation for drug development to create new therapies for MDS.
Specific Aims	Aim 1. Identify shared targets of mutant SF3B1, SRSF2, and U2AF1 that drive MDS. Aim 2. Identify compounds that inhibit mutation-dependent splicing abnormalities.
Role	Principal Investigator
Overlap	None

Title	U2AF1 mutations in myelodysplastic syndromes: from mechanism to therapy (R56 DK103854)
Effort	2.40 CPM
Agency	NIDDK
Grants Officer	Carolyn Kofa Email: kofac@extra.niddk.nih.gov Phone: (301) 594-7687
Period	09/04/14 – 11/30/15
Funding	(total costs for year 2)
Goals	We propose to determine the mechanistic, functional, and therapeutic consequences of mutations affecting the spliceosomal gene U2AF1, one of the most commonly mutated genes in MDS. We have built a team with experience in RNA splicing mechanisms and splicing-based therapeutics, as well as MDS biology and patient care.
Specific Aims	Aim 1. To determine how mutations alter U2AF1's normal role in 3' splice site recognition. Aim 2. To test the hypothesis that U2AF1 mutations induce mis-spliced protein isoforms that contribute to molecular abnormalities characteristic of MDS cells. Aim 3. To test the hypothesis that chemical inhibition of 3' splice site recognition will selectively kill cells with U2AF1 mutations
Role	Principal Investigator
Overlap	None

RECENTLY COMPLETED

Title	Repeat derepression and RNA-mediated toxicity in FSHD (P01 NS069539-06)
Effort	2.70 CPM
Agency	NINDS
Grants Officer	Glen Nucholls Email: glen.nuckolls@nih.gov Phone: (301) 496-5876
Period	09/30/15 – 09/29/20
Funding	
Goals	The broad and long-term goal of this project is to identify molecular pathways downstream of D4Z4 derepression that may be targeted to slow disease progression or improve muscle function. The major hypothesis of this project is that repetitive and other aberrant RNAs contribute to DUX4 cytotoxicity and modify FSHD severity. The specific goal of the project is to identify the mechanistic origins of aberrant RNA production and cytotoxicity, and determine whether these toxic RNAs modify FSHD penetrance
Specific Aims	Aim 1: Determine the molecular mechanisms of DUX4-mediated inhibition of RNA surveillance. Aim 2: Determine the subset of DUX4-induced RNAs that are actively translated, and test whether these aberrant RNAs produce abnormal proteins or novel peptides in DUX4-expressing cells. Aim 3: Determine whether genetic variation influences repetitive RNA expression to modify FSHD penetrance.
Role	Project Principal Investigator

Overlap	None

Title	U2AF1 mutations in myelodysplastic syndromes: from mechanism to therapy (R01 DK103854)
Effort	3.00 CPM
Agency	NIDDK
Grants Officer	Diana T Ly Email: dianaly@mail.nih.gov Phone: (301) 594-9249
Period	12/01/15 – 11/30/20 (NCE)
Funding	(total costs for year 4)
Goals	The goal of this project is to determine how myelodysplasia-associated mutations in U2AF1 alter the RNA splicing process to contribute to MDS pathophysiology. There is no research or budgetary overlap between this grant and the Evans proposal.
Specific Aims	Aim 1: Determine the mechanistic basis and consequences of the observed genetic spectrum of *U2AF1* mutations. Aim 2: Determine how *U2AF1* mutations dysregulate downstream molecular pathways, contributing to molecular features of dysplastic cells. Aim 3: Identify potential therapeutic opportunities for targeting *U2AF1*-mutant cells.
Role	Principal Investigator
Overlap	There is scientific overlap between Aims 2 and 3 of R01 DK103854 and Aims 2 and 3 of the Leukemia & Lymphoma Society Scholar Award. 3.00 CPM is concurrent between the two.

Title	Leveraging the minor spliceosome to understand and treat myelodysplastic syndromes
Effort	0.60 CPM
Agency	Evans Foundation
Grants Officer	Michael Lewis Email: mdl@epefoundation.org Phone: (978) 494-6009
Period	09/01/18 – 08/31/20
Funding	
Goals	In this proposal, we will combine experimental and computational tools to determine how a common genetic change gives rise to MDS. Mutations affecting the gene ZRSR2, which encodes a protein involved in a molecular process called RNA splicing, are common in MDS. Although ZRSR2 mutations occur frequently, it is not known why they prevent normal blood production and give rise to MDS. We will determine how ZRSR2 mutations drive the development of MDS and search for new ways to specifically target and kill cells carrying ZRSR2 mutations. The long-term goals of this research are to further our understanding of basic MDS biology and identify potential new therapies for this disease.
Specific Aims	Aim 1. Identify molecular pathways that connect *ZRSR2* mutations to dysplastic hematopoiesis. Aim 2. Identify therapeutic opportunities to selectively target *ZRSR2*-mutant cells.
Role	Principal Investigator
Overlap	None

Title	Preliminary data for the genetic origins and molecular stratification of DUX4-expresing cancers (Restricted Donation)
Effort	0.24 CPM (no salary)
Agency	IIRC, Bezos, Miguel and Jacklyn
Grants Officer	Sheila Charles (Program Operations Director) Email: scharles@fredhutch.org Phone: (206) 667-6438
Period	01/01/20 – 12/31/20
Funding	(total costs for year 1)
Goals	We recently reported that a subset of cancers suppress MHC class I-dependent antigen presentation by reactivating the expression of the early embryonic transcription factor DUX4, which is significantly associated with failure of immune

	checkpoint blockade therapy. Here, we propose to generate critical preliminary data for each of the three aims of our dual PI R01 application. Overall, the R01 application seeks to establish the knowledge and resources necessary to determine whether DUX4 suppression will improve responses to immune modulating therapies.
Specific Aims	Aim 1: Identify trans-acting somatic mutations that promote DUX4 expression in cancer. Aim 2: Determine the scope of long-term protein suppression by DUX4. Aim 3: Develop biomarkers for identifying DUX4-expressing cancers.
Role	Principal Investigator
Overlap	None

CURRENT SUPPORT

Title	The biological and therapeutic consequences of SF3B1 mutations in myelodysplastic syndromes (Career Development Scholar Award)
Effort	3.66 CPM
Agency	Leukemia & Lymphoma Society
Grants Officer	Lee Greenberger Email: researchprograms@lls.org
Period	07/01/17 – 06/30/22
Funding	(total costs year 5)
Goals	The studies proposed will combine experimental and computational techniques to determine the functional consequences of SF3B1 mutations and identify therapeutic opportunities for selectively killing SF3B1mutant cells.
Specific Aims	Aim 1: Determine the mechanistic consequences of SF3B1 mutations for RNA splicing. Aim 2: Identify molecular pathways that connect SF3B1 mutations to dysplastic hematopoiesis. Aim 3: Identify therapeutic opportunities to selectively target SF3B1mutant cells.
Role	Principal Investigator
Overlap	None.

NEWLY ACTIVE

Title	Genetic and molecular basis for SRSF2 mutations in myelodysplasia (R01 HL128239)
Effort	1.47 CPM
Agency	NIH
Grants Officer	Taryn Cobb (Grants Management Officer) Email: cobbt@mail.nih.gov Phone: (301) 827-8025
Period	08/01/2015 – 07/31/2024
Funding	(total costs for year 1)
Goals	Myelodysplastic syndromes (MDS) are a heterogeneous group of blood diseases, all of which are characterized by ineffective production of blood by the bone marrow. Here, we will investigate why mutations in the SRSF2 gene, which are commonly found in MDS, cause molecular changes within blood cells that result in ineffective blood production. We also seek to find new ways to treat MDS with SRSF2 mutations.
Specific Aims	Aim 1: Determine the molecular basis and functional consequences of widespread intron retention in SRSF2-mutant MDS Aim 2: Determine the biological and molecular basis for allele-specific interactions between SRSF2 mutations and additional genetic alterations in MDS Aim 3: Identify and test therapeutic strategies for targeting cells with spliceosomal gene mutations.
Role	Principal Investigator
Overlap	None

Title	Functional and molecular basis of ineffective erythropoiesis in SF3B1-mutant myelodysplastic syndromes (R01 HL151651)
Effort	1.80 CPM
Agency	NHLBI
Grants Officer	Taryn Cobb (Grants Management Officer) Email: cobbt@mail.nih.gov Phone: (301) 827-8025
Period	06/01/20 – 05/31/24
Funding	(total costs for year 2)
Goals	Myelodysplastic syndromes (MDS) are acquired disorders marked by clonal expansion of aberrantly differentiating hematopoietic stem and progenitor cells (HSPCs). Here, we seek to systematically identify the functional and therapeutic implications of SF3B1 mutations in MDS. We propose to determine how SF3B1 mutations alter the RNA splicing process, identify downstream splicing events that cause ineffective erythropoiesis and ring sideroblast formation, and develop novel, mechanism-based therapeutic approaches.
Specific Aims	Aim 1. Define the molecular consequences of *SF3B1* mutations for mRNA splicing, stability, and translation. Aim 2. Determine the functional basis of ring sideroblast formation and ineffective erythropoiesis in *SF3B1*-mutant MDS-RS. Aim 3. Identify therapeutic opportunities for treating MDS-RS with *SF3B1* mutations.
Role	Co-Principal Investigator
Overlap	None

Title	Defining and therapeutically targeting the immunological landscape of SF3B1-mutant breast cancer
Effort	0.90 CPM
Agency	DoD Breast Cancer
Grants Officer	Elfreda Nymn Email: elfreda.r.nymn.civ@mail.mil Phone: (301) 619-7150
Period	07/01/20 – 06/30/23
Funding	(total costs for year 2)
Goals	We hypothesize that SF3B1-mutant breast cancer cells express MHC class I-restricted novel epitopes that are targeted by an endogenous T cell response. Our objectives are to identify novel epitopes presented by SF3B1-mutant breast cancers and test the feasibility of indirectly or directly targeting these epitopes with immune checkpoint blockade and/or neoantigen-specific T cells.
Specific Aims	Aim 1. Determine how SF3B1 mutations alter the immunopeptidome by triggering neoepitope production. Aim 2. Assess the immunological consequences of SF3B1 mutations and their potential as immunotherapeutic targets in SF3B1-mutant breast cancer
Role	Principal Investigator
Overlap	None

Title	Loss of the non-canonical BAF complex as a driver and therapeutic target in SF3B1-mutant MDS and leukemia (Blood Cancer Discoveries Grant)
Effort	2.40 CPM
Agency	Leukemia and Lymphoma Society
Grants Officer	Lee Greenberger (Chief Scientific Officer) Email: researchprograms@lls.org
Period	07/01/20 – 06/30/23
Funding	(total costs for year 2)
Goals	Our recent study established that SF3B1-mutant malignancies exhibit disrupted ncBAF function that confers a competitive advantage, but it remains unknown how ncBAF disruption dysregulates gene expression, how ncBAF acts as a tumor suppressor, and whether we can prevent ncBAF disruption in SF3B1-mutant hematopoietic malignancies. We hypothesize that ncBAF is a critical mediator of SF3B1-mutant MDS and leukemia that can be exploited for therapeutic purposes.

Specific Aims	Aim 1. Determine the functional basis and biological consequences of BRD9 suppression in SF3B1-mutant hematopoietic malignancies Aim 2. Identify therapeutic opportunities for treating SF3B1-mutant hematopoietic malignancies.
Role	Principal Investigator
Overlap	None

Title	Interrogating the minor spliceosome to understand and treat leukemia
Effort	1.16 CPM
Agency	NIH
Grants Officer	Juraj Bies (Scientific Review Officer) Email: biesj@mail.nih.gov Phone: (301) 435-1256
Period	07/03/20 – 06/30/25
Funding	(total costs for year 2)
Goals	Leukemias are cancers in which the bone marrow produces too many abnormal blood cells at the expense of normal blood cells. Here we will determine how a commonly occurring change in a gene called ZRSR2, which encodes a protein that participates in the process of RNA splicing, gives rise to leukemia. We will determine how ZRSR2 mutations drive leukemia and find new ways to treat leukemias with ZRSR2 mutations.
Specific Aims	Aim 1: Determine how ZRSR2 mutations dysregulate the transcriptome and proteome in leukemia. Aim 2: Determine how disruption of ZRSR2-regulated splicing events drives clonal advantage. Aim 3: Identify the functional basis for the frequent co-occurrence of ZRSR2 and TET2 mutations in leukemia.
Role	Principal Investigator
Overlap	None

Title	Dr. Robert Bradley's Research (Donation)
Effort	0.00 CPM
Agency	Fred Hutchinson Cancer Research Center
Grants Officer	Angela Bush Email: abush@fredhutch.org
Period	11/01/20 – 10/31/21
Funding	(total costs)
Goals	The goal of this project is to use CRISPR/Cas9 to manipulate 3' UTR usage and function in cancers.
Specific Aims	N/A
Role	Principal Investigator
Overlap	None

Title	Modulation of RNA splicing to enhance immunotherapy (Technology Development Grant)
Effort	0.00 CPM
Agency	Washington Research Foundation
Grants Officer	Katie Smolnycki Email: ksmolnyc@fredhutch.org
Period	08/01/2021 – 07/31/2022
Funding	(total costs)
Goals	The goal of this project is to develop novel splicing modulatory compounds to enhance immunotherapy.
Specific Aims	N/A
Role	Principal Investigator
Overlap	None

PENDING SUPPORT
None.

NO LONGER PENDING

Title	Genetics origins and molecular stratification of DUX4-expressing cancers (R01 CA251697)
Effort	2.40 CPM
Agency	NIH
Grants Officer	Juraj Bies (Scientific Review Officer) Email: biesj@mail.nih.gov Phone: (301) 435-1256
Period	07/01/20 – 06/30/25
Funding	(total costs for year 1)
Goals	The broad and long-term goal of this project is to establish the foundation necessary to determine whether suppressing DUX4 expression will improve the clinical response to immune checkpoint blockade. The major hypothesis is that cancer cells avoid immune surveillance by re-activating DUX4 and an early embryonic program that suppresses antigen presentation. The specific aims will identify the genetic origins of DUX4 expression in cancer, discover molecular mechanisms underlying DUX4-mediated suppression of antigen presentation, and establish the foundation to support the development of DUX4-targeted therapeutics.
Specific Aims	Aim 1: Determine the somatic mutational and inherited genetic basis of DUX4 expression in cancer. Aim 2: Determine the scope of long-term protein suppression by DUX4 and the molecular mechanisms of DUX4-mediated suppression of immune recognition. Aim 3: Develop biomarkers for identifying DUX4-expressing cancers and validate platforms for drug discovery.
Role	Principal investigator
Overlap	None

Title	Functional investigation and therapeutic targeting of SF3B1-mutant uveal and mucosal melanoma (W81XWH-19-MRP-IA)
Effort	0.60 CPM
Agency	DoD Melanoma
Grants Officer	Christopher L. Meinberg Email: help@eBRAP.org Phone: (301) 682-5507
Period	09/01/20 – 08/31/23
Funding	(total costs for year 1)
Goals	We hypothesize that BRD9 mis-splicing and subsequent loss of ncBAF are critical mediators of the protumorigenic effects of recurrent SF3B1 mutations in uveal and mucosal melanomas. Our objectives are to determine how ncBAF loss dysregulates gene expression to promote melanomagenesis, and identify specific agents that correct BRD9 mis-splicing for therapeutic targeting of SF3B1-mutant melanomas.
Specific Aims	Aim 1. Determine the functional basis and biological consequences of BRD9 suppression in SF3B1- mutant uveal and mucosal melanoma. Aim 2. Identify therapeutic opportunities for treating SF3B1-mutant uveal and mucosal melanoma.
Role	Principal Investigator
Overlap	None

OVERLAP
None.

CURRENT, PENDING, AND PREVIOUS SUPPORT

GUENTHOER, JAMIE PH.D.

Changes are highlighted in yellow

PREVIOUS SUPPORT

Title	Seattle Cancer Consortium Breast SPORE (P50 CA138293)
Effort	3.00 CPM
Agency	NIH
Grants Officer	Justin Birken (Scientific Review Officer) Email: birkenjg@mail.nih.gov
Period	09/15/10 – 08/31/17(NCE)
Funding	(annual direct costs)
Goals and Aims	The Seattle Cancer Consortium (SCC) Breast SPORE includes four projects and four cores. Project 1 applies basic discovery of p27kip1 cell cycle regulation in breast cancer to predict mortality and response to therapy. Project 2 uses engineered central memory T cells to target abnormally expressed tumor-associated proteins with vaccines and therapy. Project 3 aims to determine the biological basis for a breast imaging metabolism/perfusion mismatch profile that predicts poor prognosis and poor response to systemic therapy. Project 4, draws on a well-characterized population-based cohort to identify specific DNA damage pathway biomarkers that could prevent the over, or under, treatment of women with breast cancer.
Role	Principal Investigator
Overlap	None

Title	Advancing our understanding of the etiologies and mutational landscapes of basal-like, luminal A, and luminal B breast cancers (W81XWH-12-1-0079)
Effort	0.60 CPM
Agency	DoD
Grants Officer	Cheryl Lowery Email: cheryllowery@amedd.army.mil Phone: (301) 619-7150
Period	09/25/13 – 08/31/16
Funding	(annual direct costs)
Goals and Aims	Study aims to address the existing research gaps regarding the etiologies of different molecular subtypes of breast cancer.
Role	Staff Scientist

Title	Breast SPORE Core B
Effort	5.40 CPM
Agency	Fred Hutchinson Cancer Research Center Institutional Support
Grants Officer	Marc Russell (Corporate Financial Analyst) Email: mwrussell@fredhutch.org Phone: (206) 667-6740
Period	07/01/18 – 06/30/19
Funding	(annual direct costs)
Goals and Aims	The major goal of this project is salary support for Porter Lab personnel.
Role	Staff Scientist

Title	Understanding and preventing breast cancer disparities in Latinas (P50 CA148143)
Effort	0.60 CPM
Agency	DoD
Grants Officer	Shobha Srinivasan
Period	05/01/10 – 01/31/16 (NCE)
Funding	(annual direct costs)
Goals and Aims	The four projects and four cores of this P50 application focus researchers from several disciplines on the overarching theme: to understand and prevent pre-cursors

	of breast cancer and reduce breast cancer morbidity and mortality among Latinas.
Role	Co-Investigator
Overlap	None

Title	Pharmacodynamic and Tissue Measures of Early Breast Cancer Endocrine Sensitivity
Effort	0.24 CPM
Agency	Susan G. Komen for the Cure
Grants Officer	Jamie Stanford
Period	10/01/11 – 09/09/15 (NCE)
Funding	(annual direct costs)
Goals and Aims	This proposal will examine endocrine sensitivity and mechanisms of resistance in early-stage ER+ breast cancer by pairing molecular imaging data with Ki-67 indexes and expression profile data from patients undergoing a brief round of pre-operative AI therapy. The required methods are patient selection, collection of PET images and tissue samples, IHC and microarray and PCR analysis of tissue samples, and statistical analysis of the data.
Role	Co-Investigator
Overlap	None

Title	Partnership for the Advancement of Cancer Research: NMSU-FHCRC (2 of 2) (U54 CA132381)
Effort	0.60 CPM
Agency	NIH
Grants Officer	Funmi Elesinmogun, Email: elesinmf@mail.nih.gov
Period	09/25/13 – 08/31/16
Funding	(annual direct costs)
Goals and Aims	The overall goal of this collaboration between New Mexico State University (NMSU), a minority-serving institution, and the Fred Hutchinson Cancer Research Center (FHCRC), a comprehensive cancer center, is to expand our current regional cancer program to increase knowledge and attention to cancer-related health disparities among disadvantaged populations.
Role	Co-Investigator
Overlap	None

Title	Clinical Relevance of a Novel Form of the MYC Oncoprotein in Breast Cancer
Effort	0.60 CPM
Agency	Safeway Foundation
Grants Officer	Lisa McInnis Email: lmcinnis@fredhutch.org Phone: (206) 667-6817
Period	11/01/14 – 04/30/18 (NCE)
Funding	(annual direct costs)
Goals and Aims	The overall goal of the pilot project is to assess the clinical relevance of a novel mechanism that augments tumor cell survival and motility.
Role	Co-Principal Investigator
Overlap	None

Title	Tumor-resident “badophages” in immunosuppressive microenvironments of aggressive breast cancer subtypes
Effort	4.20 CPM
Agency	Breast Cancer Research Program, Funded by the Safeway Foundation
Grants Officer	Lisa McInnis (Senior Sponsored Research Officer) Email: lmcinnis@fredhutch.org Phone: (206) 667-6817
Period	04/01/19 – 03/31/21
Funding	(annual direct costs)

Goals	The primary aim of this project is to identify molecular biomarkers in first primary breast tumors associated with risk of developing a subsequent contralateral breast cancer.
Specific Aims	Aim 1: assess if the presence and/or spatial distribution of Mφbad is associated with clinical outcomes in Basal-like and Luminal B BCs using our multiplexIHC Mφbad assay Aim 2: with single nucleus RNAseq (a) corroborate the melanoma-derived Mφbad transcriptional signature in triple negative BCs and/or identify a homologous Mφbad population; (b) identify a set of potential Mφbad-specific molecules, which we can target in follow-on studies in combination with chemo- or immunotherapy.
Role	Staff Scientist
Overlap	None

CURRENT SUPPORT

Title	Molecular pathoepidemiology of contralateral breast cancer (R01 CA206464)
Effort	1.20 CPM
Agency	NIH
Grants Officer	Justin Birken (Scientific Review Officer) Email: birkenjg@mail.nih.gov
Period	03/01/17 – 02/28/22
Funding	(annual direct costs)
Goals and Aims	The primary aim of this project is to identify molecular biomarkers in first primary breast tumors associated with risk of developing a subsequent contralateral breast cancer.
Role	Staff Scientist
Overlap	None

Title	Low-dose/protracted radiation exposure from Chernobyl and breast cancer (BCRF-19-131)
Effort	1.20 CPM
Agency	Breast Cancer Research Foundation
Grants Officer	Lisa Risi (Chief Operating Officer)
Period	10/01/19 – 09/30/21
Funding	(annual direct costs)
Goals and Aims	The major goal of this project is to characterize the relationship between low-dose protracted ionizing radiation (IR) exposure and breast cancer.
Role	Staff Scientist
Overlap	None

Title	Characterizing the broad antibody response to HIV superinfection (R01 AI138709)
Effort	4.2 CPM
Agency	NIH; NIAID
Grants Officer	David Mconald (Program Official) Email: david.mcdonald@nih.gov
Period	02/07/2018 – 01/31/2023
Funding	(annual direct costs)
Goals and Aims	The goal of this project is to study people who develop a broad polyclonal antibody response after superinfection/reinfection with HIV, which should present a more significant barrier to HIV escape and resistance than a monoclonal response.
Role	Staff Scientist
Overlap	None

Title	Early and Reinfection in High Risk Women (R01 HD103571)
Effort	2.4 CPM
Agency	NIH; NICHD
Grants Officer	Denise Russo (Program Official) Email: drusso1@mail.nih.gov

Period	04/01/2020 – 02/28/2025
Funding	(annual direct costs)
Goals and Aims	This research study proposes to define the specific innate effectors that play a role in whether a persistent HIV infection is established by taking a combined approach of studying this in humans and in biologically relevant cell culture systems.
Role	Staff Scientist
Overlap	None

Title	Characterizing the broad antibody response to HIV superinfection (administrative supplement) (R01 AI138709)
Effort	2.4 CPM
Agency	NIH; NIAID
Grants Officer	David Mcdonald (Program Official) Email: david.mcdonald@nih.gov
Period	06/01/2020 – 01/31/2023
Funding	(annual direct costs)
Goals and Aims	This proposal aims to comprehensively study the antibody responses to SARS COV-2 in people who had COVID 19.
Role	Staff Scientist
Overlap	None

Title	Defining and therapeutically targeting the immunological landscape of SF3B1-mutant breast cancer
Effort	0.60 CPM
Agency	DoD Breast Cancer
Grants Officer	Elfreda Nymn Email: elfreda.r.nymn.civ@mail.mil Phone: (301) 619-7150
Period	07/01/20 – 06/30/23
Funding	(total costs for year 2)
Goals	We hypothesize that SF3B1-mutant breast cancer cells express MHC class I-restricted novel epitopes that are targeted by an endogenous T cell response. Our objectives are to identify novel epitopes presented by SF3B1-mutant breast cancers and test the feasibility of indirectly or directly targeting these epitopes with immune checkpoint blockade and/or neoantigen-specific T cells.
Specific Aims	Aim 1. Determine how SF3B1 mutations alter the immunopeptidome by triggering neoepitope production. Aim 2. Assess the immunological consequences of SF3B1 mutations and their potential as immunotherapeutic targets in SF3B1-mutant breast cancer
Role	Co-Investigator
Overlap	None

PENDING SUPPORT

Title	The impact of HIV on the breast cancer tumor microenvironment (R21TW012100)
Effort	1.20 CPM
Agency	NIH/FIC
Grants Officer	
Period	07/01/21- 06/30/23
Funding	
Goals and Aims	The major goal of this project is to investigate the extent and nature of HIV-associated immune dysfunction in primary tumor tissue and blood biospecimens from HIV+ and HIV- Ugandan women with breast cancer.
Role	Co-Investigator
Overlap	None

OVERLAP

There is no current scientific or financial overlap.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

BC191163P1: Defining and Therapeutically Targeting the Immunological Landscape of SF3B1-Mutant Breast Cancer

PI: Evan W. Newell, Fred Hutchinson Cancer Research Center, WA **Budget:** $660,000

Topic Area: Breast Cancer Research Program **Mechanism:** Breakthrough Award – Funding Level 1

Research Area(s): SCS Coding **Award Status:** 01 July 2020 – 30 June 2023

Study Goals:

Our objectives are to characterize the unique immune landscape of *SF3B1*-mutant breast cancer and define its therapeutic potential.

Specific Aims:

Aim 1. Determine how SF3B1 mutations alter the immunopeptidome by triggering neoepitope production.
Aim 2. Assess the immunological consequences of SF3B1 mutations and their potential as immunotherapeutic targets in SF3B1-mutant breast cancer.

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date

FIGURE 1

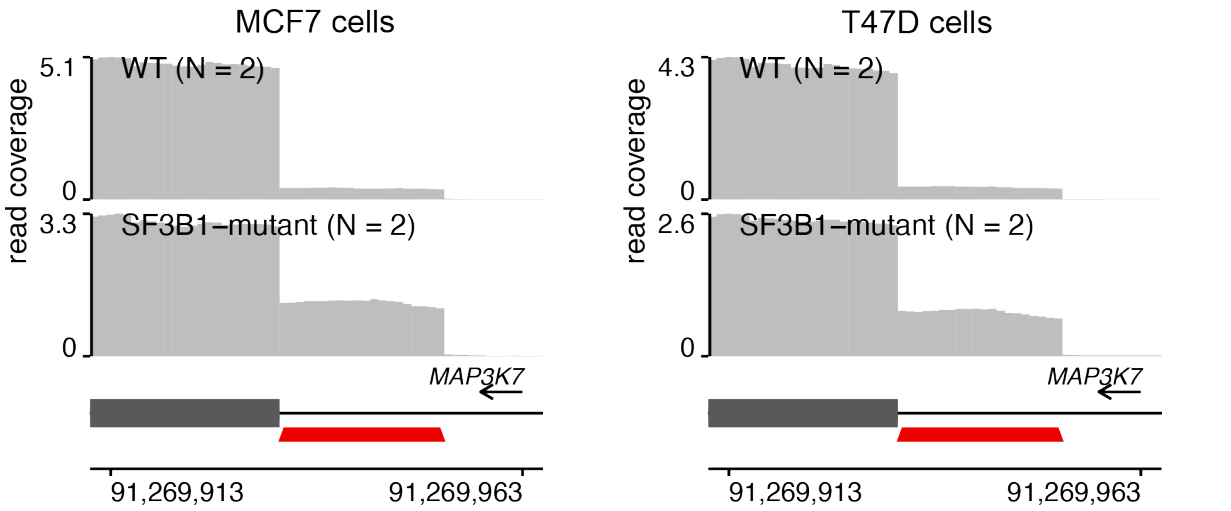

Figure 1. Mis-splicing induced by mutant SF3B1 in human breast cancer cell lines. Plots illustrate RNA-seq read coverage over a mis-splicing event (cryptic 3' splice site) in *MAP3K7* that is specifically induced by mutant SF3B1 in both MCF7 (left) and T47D (right) breast cancer cells. Genotypes correspond to dox-inducible transgenic expression of WT or mutant (*SF3B1*K700E) SF3B1. N=2 biological replicates per genotype.

FIGURE 2

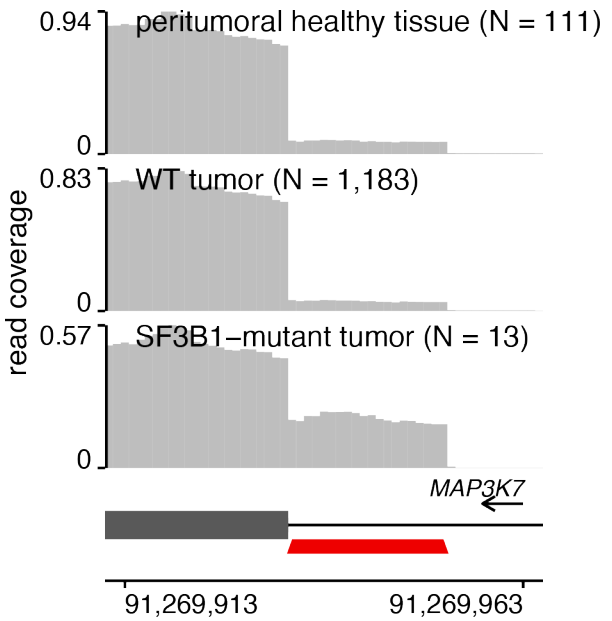

Figure 2. Mis-splicing induced by mutant SF3B1 in primary human breast cancers. Plots illustrate RNA-seq read coverage over the same mis-splicing event in *MAP3K7* event illustrated in Figure 1, but for samples from peritumoral control healthy tissue (none of which have *SF3B1* mutations), breast tumors lacking *SF3B1* mutations, or breast tumors bearing *SF3B1*K700E mutations. N indicates the number of samples per sample type and genotype. The biomarker *MAP3K7* splicing event is specifically induced by mutant SF3B1 in both primary human tumors (this figure) and in human breast cancer cell line models (Figure 1).

FIGURE 3

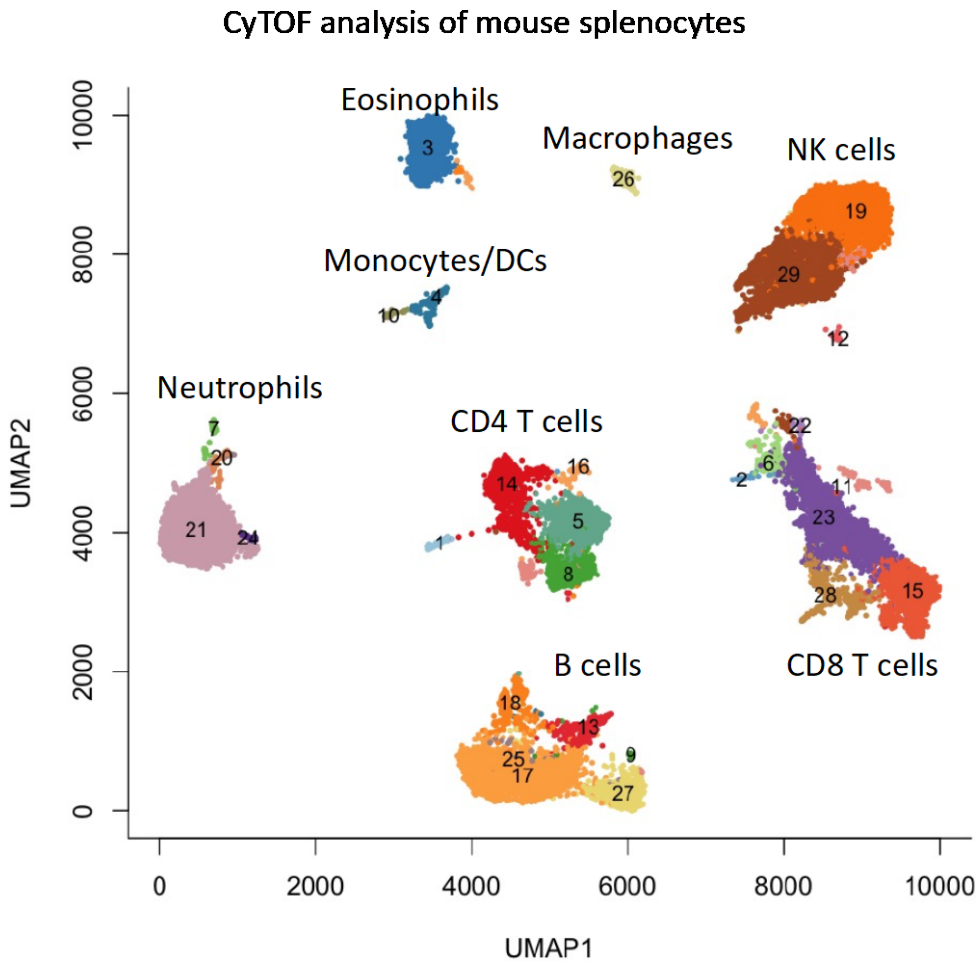

Figure 3. Optimization of mouse tumor inoculation and mass cytometry analysis panels. As part of Anthony Cessna's training in mouse handling, tumor inoculation, tissue digestion and mass cytometry analysis, this UMAP plot shows data that was collected from the analysis of mouse splenocytes using the proposed mass cytometry panel. After gating on live CD45+ single cells, the remaining 38 antibody parameters were used for UMAP dimensionality reduction and phonograph clustering. On this plot each dot represents a single cell and the cells are colored by their membership in each phonograph cluster. Based on marker expression profiles (not shown) the identities of each major cluster of cells are labeled.

FIGURE 4

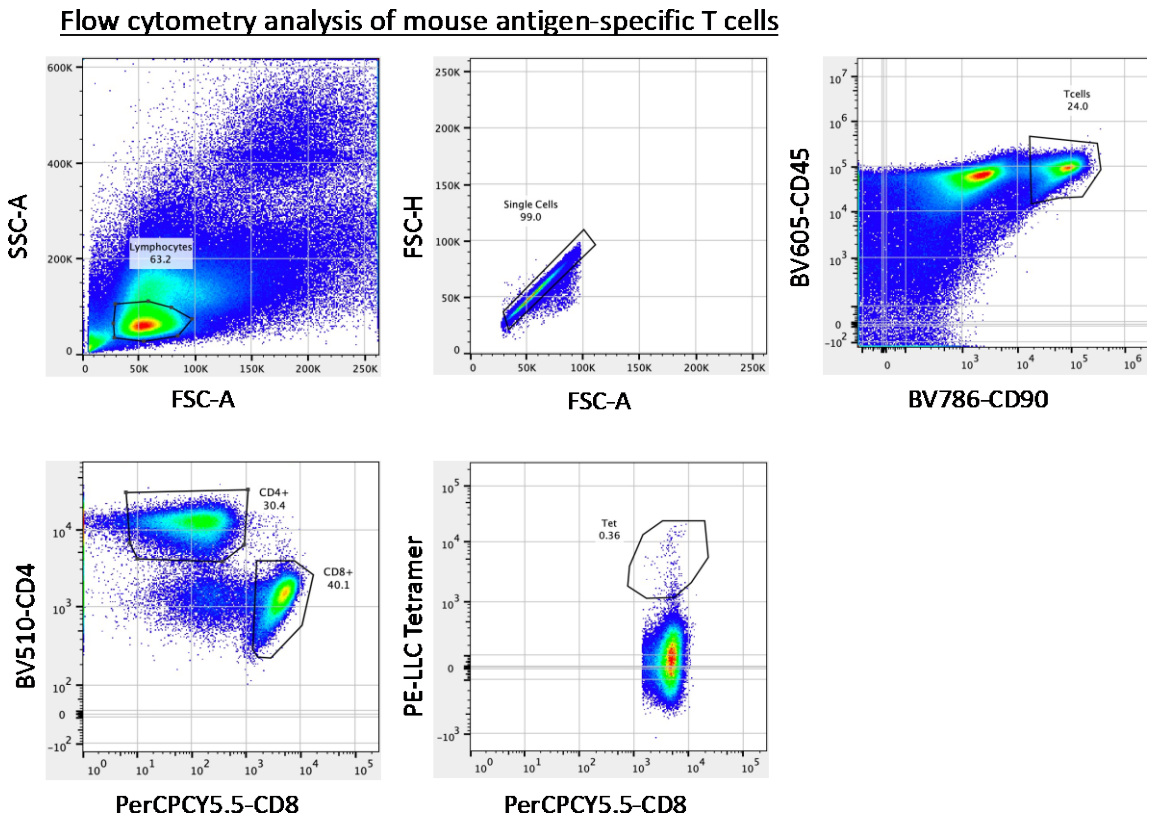

Figure 4. Testing peptide-MHC tetramer staining of in tumor specific T cells. To prepare for screening of antigen-specific cells for this project, we used the Lewis lung cancer (LLC) mouse tumor system with a known neoantigen-specific cell population to optimize staining and analysis of tumor-specific T cells. This figure shows flow cytometry analysis of mouse splenocytes from a mouse previously inoculated with the LLC mouse cell line. First lymphocytes were gated based on forward- and side-scatter then single-cells were gated based on forward-scatter area vs. height then CD45+ CD90+ T cells were identified prior to gating on CD8 and the mRiok1 (PE-LLC) tetramer.