THE DOSSIER

The Digest on CCR Staff Scientists and Staff Clinicians: Information, Employment and Research

June 2018 Issue 32

From the Editor

Welcome to the June issue of The Dossier, a newsletter dedicated to the Staff Scientists and Staff Clinicians (SSSC) of CCR!

This issue contains information on CCR's Clinical Research Program from Tom Misteli, Ph.D., and Bill Dahut, M.D., along with an overview of the Annual SSSC Retreat by the 2018 Co-Chairs Abdul Waheed, Ph.D., and Yoshimi Greer, M.D., Ph.D. In our Author's Corner, we highlight the published work of Damian Kovalovsky, Ph.D., while in our PI Corner, Vinay K. Pathak, Ph.D., discusses his research and the important role of his Staff Scientist, Krista Delviks-Frankenberry, Ph.D. Meanwhile, in our Clinical Corner, we obtain the viewpoints of Stephanie L. Goff, M.D., on several aspects of the Staff Clinician position and in our Core Corner, Valery V. Bliskovsky, Ph.D., Elizabeth A. Connor, Ph.D., and Yong-Chen William Lu, Ph.D. describe new approaches to identify antigen-specific T cell receptor sequences in the CCR Genomics Core. In our personal development corner, Brunilde Gril, Ph.D. discusses aspects of well-being, while we learn about the research of Matthew J. Anderson, Ph.D. in our SSSC Corner. We hope to continue to provide pertinent information to aid in the success of SSSCs. Please send your contributions, suggestions and comments to budhua@mail.nih.gov.

Anuradha Budhu, Ph.D. (SS)
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In This Issue

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The Office of the Director: Guest Editorial*

*The CCR Director regularly invites senior staff members as guest columnists to expertly inform the SSSC community on diverse aspects of the CCR.

Update on the CCR's Clinical Research Program

It's an incredibly exciting time to be a clinical investigator in the CCR. There is a major explosion of knowledge and real advances in the treatment of patients with cancer. We appreciate the complexity of clinical research and would like to share with you several new initiatives that we are confident will improve our clinical operations.

Career Recognition for Staff Clinicians

The importance of recognizing the critical and varied role that staff clinicians play in the NIH clinical program and their stature in their field has long been appreciated. CCR took the lead in addressing this in October 2015 with the development of a new professional titling model creating career advancement levels: Assistant Research Physician, Associate Research Physician, and Senior Research Physician. This model is very similar to the clinical scholar and clinical educator tracks common in most academic institutions. The use of professional titling in CCR was optional but we were pleased to see that many CCR staff clinicians participated.

In 2017, the CCR model was adapted by NIH with several important modifications. First, the assignment of professional designations is now mandatory throughout the NIH. To meet this requirement, CCR senior leadership recently granted a professional title to all current staff clinicians. Second, each designation now affords an additional budget allocation to support the staff clinician's research activities. In addition, CCR encourages each branch to support travel for each staff clinician to two scientific meetings annually. Third, Associate and Senior Research Physicians are now eligible to serve as the Principal Investigator (PI) on Cooperative Research and Development Agreements (CRADAs), Clinical Trial Agreements (CTAs), and/or Material Transfer Agreements (MTAs) with supervisory approval. Last, NIH requires that all initial Letters of Intent indicate the proposed staff clinician professional title and the expected time the staff clinician will spend on patient care/services and research activities in support of their PI/branch's clinical mission. NIH mandates a minimum of 50% effort be spent on clinical care or clinical research support.

To learn more about these designations and the process to apply, visit the CCR ARC website. Your branch administrative support staff and Administrative Officer can assist. Unsure of your professional designation? Contact Aubrey Wachter, Clinical Program Administrator, Office of the Director.

Strengthening Our Clinical Research Support Infrastructure

Over the past year, CCR has been at the forefront of some truly impactful clinical discoveries, including the FDA approval of avelumab for a rare skin cancer, multiple advances in immunotherapy and improved imaging of localized and metastatic prostate cancer to name only a few. Looking ahead, we expect multiple significant clinical research advances to add to these accomplishments in the coming year. We salute the many staff clinicians and staff scientists, who have been critical parts of these advances.

The CCR currently has 418 active protocols under IRB oversight. Our inpatient and outpatient census numbers are increasing. Over the last decade, the numbers of CCR-held Investigational New Drugs (INDs) have increased dramatically. Currently, CCR is the sponsor of 88 active INDs; approximately half filed with Center for Biologics Evaluation and Research (CBER) and half with Center for Drug Evaluation and Research (CDER). There are 120 protocols filed under those INDs. In addition, CCR has filed two Drug Master Files with the FDA. Given these extensive activities, we are continuing to strengthen our clinical infrastructure in support of this vibrant clinical research program and to meet the increasing regulatory requirements associated with human subjects research.

NIH policy now requires that all INDs must be held centrally within each IC. We are currently recruiting for a Medical Director to lead a new Office for Sponsored Clinical Research, which will be focused on oversight of CCR-held INDs. The sponsor of an IND has significant regulatory obligations, including developing and submitting IND applications using the electronic Common Technical Document (eCTD) format; Safety Monitoring and Oversight, which includes a robust pharmacovigilance program and medical monitoring, local site monitoring, site initiation and close

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out visits; product management; Good Manufacturing Practices (GMP); and Good Laboratory Practices (GLP). In fact, the PI's role and the role of the sponsor are significantly different. The sponsor communicates directly with the FDA about the investigational product with input from the PI. By integrating early in the development process, we expect to improve the quality of our work and expedite and simplify the FDA review and eventual approval of our IND's, strengthening our position as a national leader in the conduct of clinical research.

We have been working on multiple fronts to build our capability for cGMP processing in support of our immunotherapy research. This is a challenging, costly and complicated process, but we are making progress. The Clinical Center's Department of Transfusion Medicine (DTM) has informed us that all renovations on 2J are now complete. Efforts are now being made to rapidly acquire skilled staff to enable all rooms to become operational. We are investigating partnerships with the Clinical Center to improve the efficiency of the cell therapy process in a way that is safe and beneficial to patients. We also are working in partnership with the Division of Cancer Treatment & Diagnosis (DCTD) to leverage facilities and resources in Frederick to support cGMP.

CCR is pleased to welcome Ken Aldape, M.D., as the new Chief of the Laboratory of Pathology. Ken will be leading a major acceleration of molecular profiling of tumors in collaboration with the Genetics Branch. Please reach out to Ken about any pathology-related research questions you are interested in. We also welcome Eytan Ruppin, M.D., Ph.D., as the first Chief of the new Cancer Data Science Laboratory (CDSL). The CDSL is housed on the first floor of the Clinical Research Center and Eytan is very interested in working with clinical researchers to interrogate clinical and correlative data to increase understanding and maximize clinical insights.

Thank You

As in all organizations, we face challenges. Yet we are fortunate in CCR to have access to the Clinical Research Center, spectacular technology, exciting research ideas driven by incredibly powerful multidisciplinary teams and an exceptional workforce. The CCR clinical program is unique and impactful and we are truly making a difference in the lives of our patients and future generations. I thank each of you for your commitment and dedication to the safety and care of our patients, and to our research. It would not be possible without you.

Bill Dahut, M.D.
Scientific Director for Clinical Research/Clinical Director, CCR

Tom Misteli, Ph.D.
Director, CCR

The 14th Annual SSSC Retreat

The 14th annual Staff Scientists and Staff Clinicians (SSSC) Retreat was held on 6th April 2018, at NCI Shady Grove. This year's retreat was sponsored by NCI's Center for Cancer Research (CCR), Division of Cancer Epidemiology and Genetics (DCEG), and Frederick National Laboratory for Cancer Research (FNLCR). The theme of this year's retreat was "*Cutting Edge in Cancer Research: Cancer Immunotherapy*", a topic selected following a survey conducted among the SSSC. Given the renewed focus on Cancer Immunotherapy, particularly at NCI, it was an outstanding opportunity to hear about this cutting-edge cancer research area from experts in this field. The retreat was attended by over 160 participants from CCR, DCEG, and Leidos who shared their science, networked and were inspired by impressive speakers.

Steven Rosenberg, M.D., Ph.D., delivering the keynote lecture at the 2018 SSSC Retreat.

The retreat began with a welcome address by the retreat Co-Chairs and an introduction of the committee members, followed by warm opening remarks from Montserrat Garcia-Closas, M.D., Dr.P.H., Deputy Director, DCEG. The keynote speaker, Steven Rosenberg, M.D., Ph.D. (Chief, Surgery Branch, CCR) began the retreat's scientific session with an outstanding presentation on the topic "Lymphocytes as a drug for the treatment of cancer". Following the keynote lecture, there were four talks from distinguished panelists who are prominent leaders in the field. The panelists were introduced by Claudia Palena, Ph.D. (Laboratory of Tumor Immunology and Biology, CCR). Nicholas Restifo, Ph.D. (Surgery Branch, CCR) covered some of the basic aspects of cancer immunotherapy and T cell-based cancer immunotherapy. Ira Pastan, M.D. (Laboratory of Molecular Biology) spoke on Mesothelin, a tumor differentiation antigen: its discovery, function and target for cancer therapy. James Hodge, Ph.D. (Laboratory of Tumor Immunology and Biology, CCR) highlighted the importance of combination immunotherapies. Louis Weiner, Ph.D. (Georgetown University) focused his talk on new insights into molecular mechanisms of resistance to immunotherapy. After the short talks from the panelists, the panel discussion was moderated by Dr. Palena. It was the highlight of the retreat with a spirited question and answer session among the panelists and the audience. At the end of the panel discussion, all participants got together for a group picture along with the speakers.

The afternoon session consisted of two poster sessions and oral presentations from selected abstracts. There were 86 abstracts submitted this year, and each abstract was reviewed by three independent PIs from NCI to select the top five abstracts for oral presentation. The abstract team of the retreat committee took the responsibility of selecting the five best abstracts for oral presentation. The oral presentations were given by Krista Delviks-Frankenberry, Ph.D., Yanlin Yu, Ph.D., Jason Stagno, Ph.D., Uma Shankavaram, Ph.D., and Murali Palangat, Ph.D. These presentations from SSSC were moderated by Balamurugan Kuppusamy, Ph.D. All abstracts were presented as posters, and this year each poster session was extended for one hour. Over 90 posters were presented, and poster presentations provided an opportunity for SSSC to present their work and network. To evaluate the best posters, the poster team of the retreat committee recruited several judges from NCI and each of the top-ranked abstract-posters were judged by three independent experts in their respective fields. Following oral presentations, updates from the various SSSC organization sub-committee chairs were provided, including Emily Tai, Ph.D. (elected SSSC Co-Chair), Anuradha Budhu, Ph.D. (communication), Christina Stuelten, Ph.D. (brown bag seminar series), Swati Choksi, Ph.D. (professional development) and Even Walseng, Ph.D. (social).

Along with travel awards for best oral and poster presentations, there was also an outstanding mentor award selected from nominations from their postbacs and interns. This year the outstanding mentor award, which started two years ago, went to Chi-Ping Day, Ph.D., and the award was presented by Jonathan Wiest, Ph.D., Director, Center for Cancer Training, NCI. The travel awards were presented by Ethen Dmitrovsky, M.D., Director, Frederick National Laboratory for Cancer Research. The travel award for the best oral presentation was given to Krista Delviks

The 14th Annual SSSC Retreat Con't

The panel discussion at the 2018 SSSC Retreat: from left to right are Claudia Palena, Ph.D., Nicholas Restifo, Ph.D., James Hodge, Ph.D., Ira Pastan, M.D., and Louis Weiner, Ph.D.

-Frankenberry, Ph.D., (Retroviral Replication Laboratory, HIV Dynamics and Replication Program, CCR) for her outstanding presentation. The recipients of the travel award for best posters were: Nicolas Cuburu, Ph.D., (Laboratory of Cellular Oncology, CCR), Patricia Day, Ph.D. (Laboratory of Cellular Oncology, CCR) and Adam Cheuk, Ph.D. (Cancer Genetics Branch, Leidos).

The closing remarks were given by Glenn Merlino, Ph. D. (Scientific Director for Basic Research, CCR), He reiterated the mission and vision of NCI to improve the lives of cancer patients by leading cancer research. He also discussed the scientific accomplishments and the goals we have in cancer treatment. He congratulated us for our commitment to high-quality research and the role we play in mentoring future generation of scientists.

We are thankful as always to Dr. Wiest, for his tremendous support and assistance from Nicole Garner and Angela Jones with the planning and organization of the conference. We also thank Ted McCutchen for the conference website. We thank all judges who judged abstracts and especially those who made a trip to Shady Grove to judge posters. We thank all members of the organizing committee for their hard work and support in organizing the SSSC Retreat and they include Kajal Biswas, Ph.D., Paul Boyer, Ph.D., Ravindra Chalamalasetty, Ph.D., David Danforth, Ph.D., Siddhartha Datta, Ph.D., Shannon Doyle, Ph.D., Sigrid Dubois, Ph.D., Duane Hamilton, Ph.D., Michael Kruhlak, Ph.D., Balamurugan Kuppusamy, Ph.D., Zhihui Liu, Ph.D., Ruibai Luo, Ph.D., Vladimir Majerciak, Ph.D., Prashant Mishra, Ph.D., Anu Puri, Ph.D., Shree Ram Singh, Ph.D., Arthur Shaffer, Ph.D., Sergey Tarasov, Ph.D., Wanping Xu, Ph.D. We thank all the attendants who participated in this retreat and shared their research. We would like to take this opportunity to invite new SSSC to join the SSSC retreat committee. Your creative thoughts and ideas will improve the retreat.

Abdul Waheed, Ph.D. (SS)

Yoshimi Greer, M.D., Ph.D. (SS)

Co-Chairs, SSSC Retreat 2018

Philosophical and Scientific Perspectives of Well-Being

We all want happiness, well-being, enjoyment, and good quality of life. These are very common and overlapping objectives and yet, what do we really mean by happiness or well-being? Do we know what would lead us to everlasting contentment and joy? Is there one single definition fitting everybody? Are there any cultural differences?

From the antiquity to present days, philosophers, writers, spiritual leaders, and scientists have deciphered, analyzed, and conceptualized the construct of well-being. Rooted in Greek philosophy, two types of well-being have survived across a couple of millennia: hedonism and eudaimonia. Hedonism tends to be similar to our modern definition of happiness. It refers to immediate gratification, increase in positive emotions, and decrease in pain. Eudaimonia refers to unfolding our full potential, following virtuous principles, and being driven by a sense of meaning and purpose in life that goes beyond our self-interests. Although distinct, hedonism and eudaimonia are not mutually exclusive and can influence each other. We all experience various levels of both, but some temperaments are more conducive to one or the other. The field of positive psychology, which has blossomed over the past two decades, investigated and operationalized those two forms of well-being. Scientific studies revealed intriguing biological underpinnings distinguishing hedonism vs. eudaimonia.

In 2015, Steven W. Cole, Ph.D., a leader in the field of psychoneuroimmunology, was invited to the NIH to present his work on human social genomics. Dr. Cole showed that eudaimonia and hedonism were associated with specific gene expression patterns in the peripheral blood mononuclear cells (PBMCs)[1]. He demonstrated that individuals who defined well-being as primarily finding life meanings and goals beyond self-interest (i.e., eudemonic well-being), as opposed to people predominantly focused on immediate gratification (i.e., hedonistic well-being), presented decreased expression of proinflammatory cytokines such as IL6, IL8, and TNF and increased expression of genes involved in type I IFN antiviral responses and IgG antibody production, emphasizing the importance of a deeper and transcendent form of happiness/well-being.

In the context of our job, I would translate the hedonistic perspective as follows; I will feel happy only if my experiments confirm my hypothesis, I publish in high impact factor journals, and I have the Wednesday curry tofu of the bldg. 35 cafeteria served every day. Well, let's face it, I would set myself for disappointment. Is it realistic to have curry tofu every day? and if it were, would it still procure me unlimited joy? Fortunately, our job offers multiple opportunities to focus on unselfish life goals and pursue eudemonic flourishing. The first thing is to keep in mind the big picture of our work: to advance scientific knowledge to improve health and patient care. Often, our work is so narrowed on one specific aspect of a disease that we lose this dimension and forget the overall mission of our institution. Other daily opportunities to shift away from egocentricity reside in training the next generation of scientists, helping a student discover his passion, propelling a post-doc fellow to her 1st job, and contributing to the prosperity of the community, which can be our branch, our institute, or the scientific community at large. In summary, once we unclutter our mind from self-centered preoccupations, we have a lot of choices from which to choose our own bliss!

The hedonism/eudaimonia dichotomy has been debated, though. The perception of well-being is embedded in a cultural make-up and can differ across individuals. Interestingly, some predictors of well-being have remained the same across individualistic and collectivistic cultures[2]. The specific construct "meaning in life" has been found to be consistently associated with eudaimonia and a key aspect to define well-being worldwide, across one hundred and nine countries from seven different regions around the globe[3].

The importance of finding Meaning in Life (MIL) was pioneered and mastered by the Austrian neurologist, psychiatrist, and Holocaust survivor, Victor Emil Frankl[4], M.D., Ph.D. Since then, scholars in psychology have investigated the construct of MIL for decades, generating several conceptual models and measurements to operationalize it[5,6]. MIL is a multidimensional construct comprising three elements: comprehension, purpose, and significance[7,8]. Comprehension is the cognitive component, purpose entails motivation and action, and significance refers to the value or impact one places on his/her life. George and Park defined MIL as "*the extent to which one's*

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Section Editor: Brunilde Gril, Ph.D., M.P.S. (*SS*)

life is experienced as making sense, as being directed and motivated by valued goals, and as mattering in the world"[7]. A systematic review analyzed seventy publications investigating the relation between MIL and physical health[9]. MIL was associated with a reduction in unhealthy behaviors such as smoking, use of alcohol, or eating disorder symptoms. A greater sense of purpose in life (a subconstruct of MIL) correlated with a decrease in mortality[10,11,12] and a better sense of control over one's health[13]. Friedman et al. identified an association between well-being and a lower level of inflammatory cytokines in aging women who have a high sense of purpose in life[14].

The sources of meaning in life can be variable depending on individuals and situations encountered. Based on his professional expertise and personal experience, Dr. Frankl identified three specific ways to develop meaning in life: "*1- by creating a work; 2- by experiencing something or encountering someone; 3- by the attitude we take toward unavoidable suffering*"[4]. Finding meaning lies in our capacity to transcend our perception of mundane reality, to see beauty, goodness, knowledge, and opportunities for growth in any situations. We are always free to choose how to interpret our reality, our daily work encounters. I can be offended by a taunting Western-blot film and a mocking immunofluorescent staining disproving my hypothesis. Or, no matter the outcome of the experiments, I can choose to recall my passion for scientific discovery and feel each time an inch closer to understanding the nature of reality. We push away the confined boundaries of our self-centered universe and dismantle our personal interpretation of reality. Happiness is the epiphenomena irradiating from the perception of being part of something bigger than oneself.

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The Core Corner

Section Editor: Anne Gegonne, Ph.D. (*SS*)

An Efficient Single-Cell RNA-Seq Approach to Identify Neoantigen-Specific T Cell Receptors

The CCR Genomics Core has a long history of collaboration with the research team of Steven Rosenberg, M.D., Ph.D. in the Surgery Branch. Several technologies provided in the Core, such as NanoString, RNA-Seq and Single-Cell RNA-Seq, have been utilized to generate key data for publications over the past decade (Beard et al, *Clin Can Res*, 2013; Cohen et al, *J Clin Invest*, 2015; Tran et al, *N Engl J Med*, 2016). A recent technical challenge is how to quickly and accurately identify antigen-specific T cell receptor (TCR) sequences, which can be synthesized, characterized and potentially used in new T-cell based cancer immunotherapies. Yong-Chen William Lu, Ph.D, Staff Scientist in the Surgery Branch has been addressing this issue through performing Single-Cell RNA-Seq experiments in collaboration with Staff Scientist, Valery Bliskovsky, Ph.D., in the CCR Genomics Core. In the most recent study (Lu et al, *Molecular Therapy*, 2018), a polyclonal tumor infiltrating lymphocytes (TIL) population was co-cultured with autologous dendritic cells presenting a mutated antigen (neoantigen) library identified from a patient with metastatic colorectal cancer. The stimulated T lymphocytes were then subjected to a Fluidigm C1 Single Cell Auto Prep system to prepare single-cell RNA-seq samples. These single-cell samples were barcoded, pooled and deep-sequenced by Illumina MiSeq (Figure 1A). In Figure 1B, a full-length TCR sequence was identified based on the homology of variable gene segments and unique CDR3 (complementarity determining region 3) sequence. In this set of single-cell samples, 7 single cells expressed high levels of interferon-γ (IFN-γ) mRNA and two single cells contained detectable interleukin-2 (IL-2) mRNA. These single cells all contained the same TCRα/β sequences, and later experiments showed that this TCR recognized HLA-C*0802-restricted, mutated KRAS(G12D). The results of this study have led to a new Single-Cell RNA-Seq approach to identify neoantigen-specific TCRs with clinical as well as basic and translational implications. The collaboration with CCR Genomics Core is essential for this research and accelerates the research goals of Surgery Branch.

The CCR Genomics Core provides NCI and NIH scientific communities with access to cutting-edge genomic technologies. We are an “open access” facility with goal of providing efficiency and quality of a centralized facility with the speed and convenience of

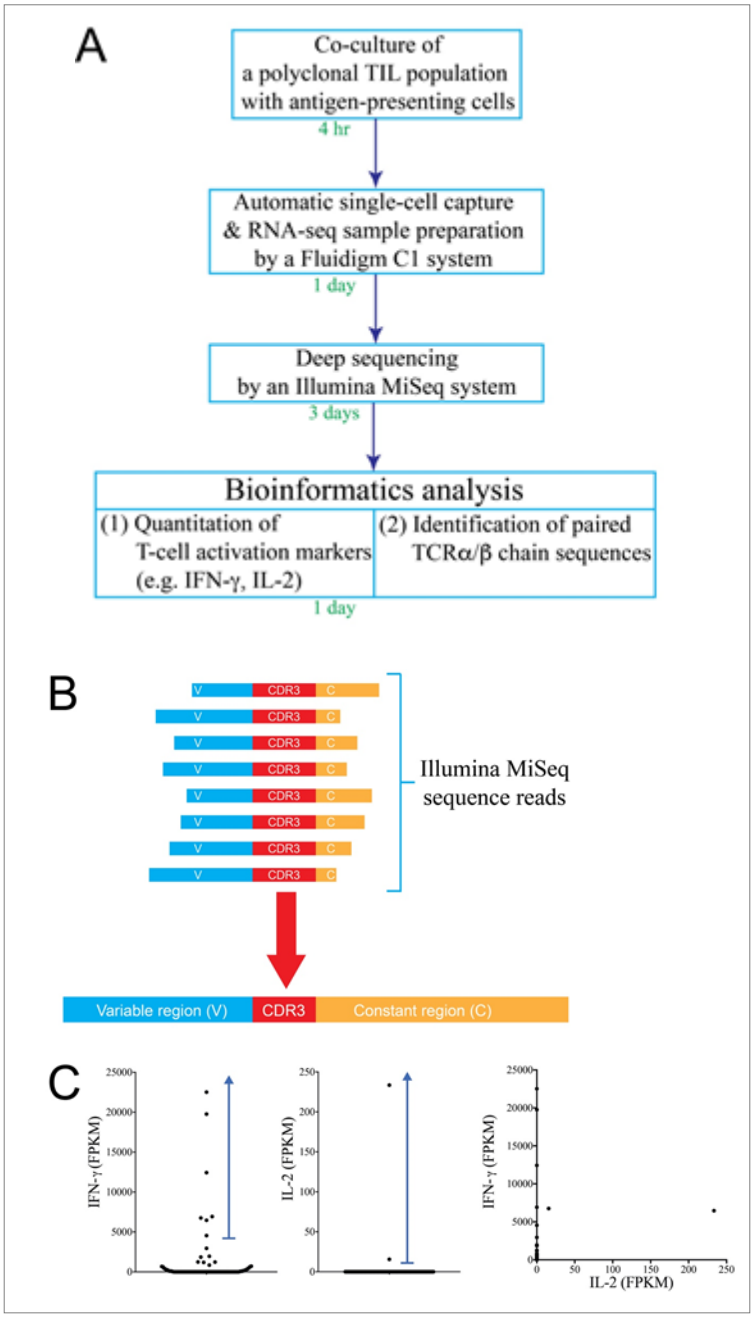

Figure 1. A single-cell approach to identify neoantigen-specific TCRs. Further details of this study can be found Lu et al, *Molecular Therapy*, 2018.

CCR Genomic Core Technologies	Sequencing
	• Sanger Sequencing
	• Next-Generation Sequencing (Illumina MiSeq, NextSeq500 & NextSeq550)
	Nanostring Digital Gene Expression Analysis
	Droplet Digital PCR Bio-Rad QX200
	Single Cell Autoprep System Fluidigm C1
	Agilent TapeStation 2200 & 4200
	Sage Science Pippin
	Qubit Fluorometric Quantitation
	Various robotic devices

Figure 2. CCR Genomics Core available technologies.

dedicated laboratory instrumentation and experienced staff. The core works closely with the Office of Science and Technology Resources (OSTR) to identify, evaluate and make available new technologies as they emerge and works in partnership with the Genome Analysis Unit (Peter Fitzgerald, Ph.D.) and the CCR Single Cell Analysis Facility (Michael Kelly, Ph.D.). The facility, headed by Elizabeth A. Conner, Ph.D., is located on the Bethesda campus in building 37, room 2135. Available Core technologies are summarized in Figure 2. Investigators can register and request services through our iLab website (https://nci.corefacilities.org/account/log-in) or visit our Core website for additional information (https://ostr.cancer.gov/node/272).

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The PI Corner

Section Editor: Lakshmi Balagopalan, Ph.D. (*SS*)

I received my Ph.D. at University of California, Davis where I worked on eukaryotic translational control mechanisms in 1988. I was a postdoctoral fellow under Howard Temin, Ph.D. who received a Nobel Prize for his discovery of reverse transcriptase, where I determined the forward mutation rate of a retrovirus and characterized several types of mutations that occur during error-prone reverse transcription. After my postdoctoral training, I started my independent research career at West Virginia University first as an assistant professor, then as an associate professor with tenure from 1991-1999. In 1999, I was recruited by John Coffin, Ph.D. to join the HIV Dynamics and Replication Program as Senior Investigator and Head of the Viral Mutation Section. We study pathogen-host interactions and seek to elucidate how HIV-1 overcomes potent inhibition by host APOBEC3 proteins. When comparing HIV-1 and HIV-2, which cause AIDS in humans, we found that they utilize different host protein degradation pathways to induce APOBEC3 degradation to counteract their antiviral activity. Krista Delviks-Frankenberry, Ph.D. (Staff Scientist in our section) and I are developing lentiviral vectors that can efficiently deliver APOBEC3 mutants to hematopoietic stem cells with the goal of developing a gene therapy treatment and functional cure for HIV-1 infection. Additionally, we are using APOBEC3 proteins as tools to fluorescently label HIV-1 complexes to gain novel insights into the dynamics of HIV-1 replication and nuclear import in living cells by using high-resolution microscopy.

Vinay K. Pathak, Ph.D.
Chief, Viral Mutation Section,
HIV Dynamics and Replication Program

The SSSC Corner

Section Editor: Takashi Furusawa, Ph.D. (*SS*)

I came to NCI-Frederick 10 years ago after finishing my PhD in Tumor Biology at Georgetown University. At Georgetown, I used zebrafish to study angiogenesis and found studying aspects of tumor biology in a developmental context to be rewarding. I therefore joined the laboratory of Mark Lewandoski, Ph.D., in the Cancer and Developmental Biology Laboratory (CDBL). My work in Dr. Lewandoski's lab focuses on FGF signaling in mouse embryonic development. We use loss and gain-of-function genetics to explore redundant and non-redundant requirements for FGFs in developmental processes that also impact cancer, such as cell death, proliferation, and signal transduction.

We recently published a study that elucidated the role of *Fgf3* in embryonic axis extension. *Fgf3* holds a special place in the history of mouse genetics as it was the first gene targeted using an exogenous selection cassette. When Mario Capecchi, Ph.D., performed this ground-breaking work (Dr. Capecchi shared the 2007 Nobel prize for gene targeting), he found that homozygous *Fgf3* mutants had two phenotypes, an inner ear malformation and a posterior axis extension defect: a curly tail. The ear defect has been extensively studied but the curly tail went mostly unexplained for over 20-years, until our work. We found

that FGF3 restricts neural crest induction in the posterior neural tube and when lost, neural crest induction expands caudally in close proximity to the closing neural tube. *Bmp4* is expressed in neural crest cells and is also a potent inhibitor of neural tube closure. Therefore, this expansion of neural crest results in a delay in the timing and extent of posterior neural tube closure, resulting in spina bifida and spina bifida occulta, both of which are common human birth defects. This work not only answered a decades old question but also brought to light a new role of FGF signaling in neural tube closure.

My mentor, Dr. Lewandoski, is a pioneer in mouse genetics with his ground-breaking work with the Cre-LoxP system, and we continue to make advances in mouse genetic tools. We have generated a number of new mouse lines including an inducible-Cre line that is getting extensive use for studying mesodermal gene function as well as other novel lines. We also strive to stay at the forefront of technological advances, including CRISPR, multiplex fluorescent mRNA in situ hybridization, and lightsheet microscopy. Recently, we decided to build our own lightsheet microsopce due to the prohibitive high price of commercially available lightsheet microscopes. We now have a lightsheet microscope for imaging large and complex embryos that costs a fraction of what the commercial models.

When not in the lab, I have two amazing children that keep me and my wife busy. We enjoy hiking the beautiful surrounding parks and visiting the wonderful nearby museums. A hobby that I have had for over 18 years is keeping a reef aquarium. In this time, I have learned a lot about coral, fish, and invertebrate care and strive to be a responsible hobbyist by using cultured corals and fish that do not contribute to the destruction of wild habitats. I enjoy the beauty of the

Dr. Anderson's reef aquarium is pictured.

aquarium as well as the learning and problem-solving that are required to maintain it. My kids also thoroughly enjoy the aquarium and it has provided an opportunity to teach them about ecology, chemistry, and animal behavior. So, I guess science is a bit pervasive in my life, and I really enjoy it. I count myself very fortunate to be at the NCI and sincerely love the work that I do and the atmosphere in which it is done.

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Zbtb1 Controls NKp46+ ROR-gamma-T+ Innate Lymphoid Cell (ILC3) Development

Ying Lu, Xianyu Zhang, Nicolas Bouladoux, Saransh Neel Kaul, Kangxin Jin, Derek Sant'Angelo, Yasmine Belkaid and Damian Kovalovsky. Oncotarget. 2017 Jul 27;8(34):55877-55888

In order to understand the transcriptional mechanisms that control hematopoietic differentiation, I decided to focus my research on a family of transcription factors, known as POK/Zbtb. Proteins of this family have a zinc finger binding domain, which recognizes specific DNA sequences, and a POZ domain that mediates homo or heterodimerization. Although POK/Zbtb proteins are characterized as transcriptional repressors, they can also function to activate transcription. What interested me most about this family was that the function of many members was unknown, and some members were starting to be identified as essential for the generation of specific immune lineages, for example, PLZF controls iNKT development, Bcl6 controls B-cell differentiation, and Th -POK determines CD4 vs CD8 lineage choice.

In particular, a recently identified mouse strain with a point mutant in Zbtb1, named "ScanT", completely lacked T-cells but presented other lymphoid and myeloid lineages. This finding was not only an opportunity to investigate how Zbtb1 was essential for T-cell development, but also to discover new functions of Zbtb1 in other immune lineages. The hypothesis was that an essential molecule may acquire other functions during evolution. Given the parallels between the differentiation of T-cells and innate-like cells (ILCs), I decided to investigate if absence of Zbtb1 led to defects in ILCs in the intestinal mucosa[1].

At first, it was observed that Zbtb1 was expressed by all ILCs at low levels, however, the generation of ILC1, ILC2 and ILC3 subsets in the intestinal mucosa was not altered in ScanT mice. I decided to dig deeper and looked if subpopulations of ILC3s were altered. ILC3s can be divided into three groups that are identified by surface markers. NKp46$^-$CCR6$^+$ILC3s are lymphoid tissue-inducer cells (LTi) that are required for the development of lymph nodes and Peyer's patches. NKp46$^-$CCR6$^-$ILC3s are the precursors of NKp46$^+$CCR6$^-$ILC3s cells, which develop after birth in response to the microbiota and Notch signals, and are characterized IFN-g secretion. Postnatal

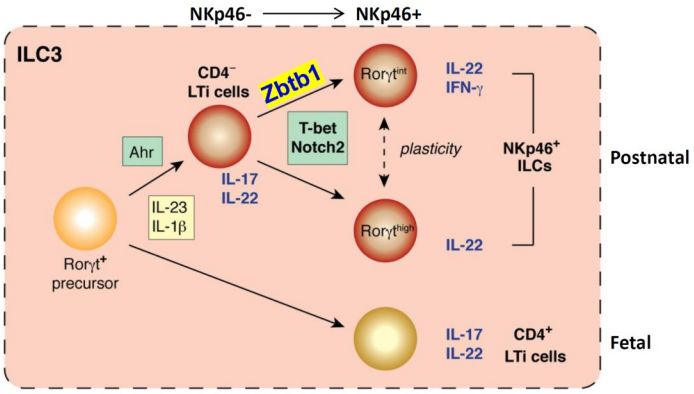

Figure 1. The role of Zbtb1 in ILC3 development (modified from Rankin *et al.* [3])

NKp46$^+$ ILC3s have a specific role in preventing inflammation and ulceration of the caecum after *C. rodentium* infections in immune competent mice and play a redundant role with other ILC3 subsets and T-cells in protecting the gastrointestinal tract. I observed that NKp46$^+$ ILC3s were severely depleted in ScanT mice, leading to absence of IFN- g secretion by ILC3s. This deficiency of NKp46$^+$ ILC3s was cell-intrinsic as hematopoietic precursors lacking Zbtb1 failed to generate NKp46$^+$ ILC3s either *in vivo*, in mixed bone marrow chimeras, or *in vitro* in co-culture with the OP9-DL1 stroma cells.

In previous work, I had identified that one of the mechanisms by which Zbtb1 affected the generation of T-cells, and other immune lineages, was by preventing DNA damage and activation of p53-mediated apoptosis in hematopoietic progenitors that are undergoing rapid proliferation[2]. This increased apoptosis was reverted in compound mice either lacking p53 or overexpressing bcl2. As NKp46$^+$ ILC3s are also generated after extensive proliferation of NKp46$^-$ ILC3s, the hypothesis was that lack of p53 or overexpression of bcl2 may also revert the NKp46$^+$ ILC3 phenotype of ScanT mice. However, compound mice also lacked NKp46$^+$ ILC3s, indicating that the

mechanism of their absence was not p53-mediated induction of apoptosis.

Finally, in collaboration with Yasmine Belkaid, Ph.D., Senior Investigator in the Mucosal Immunology Section at NIAID, we analyzed if absence of Zbtb1 in ScanT mice led to increased susceptibility to *C.rodentium* infections. We observed that ScanT mice were impaired to clear the bacteria and this led to a transient loss of body weight and shortening of the colons, indicative of increased inflammation. The transient nature of this phenotype correlates with the redundant role of ILC cells in this disease model. In summary, this work uncovered a novel function of Zbtb1 in controlling the generation of NKp46^{+} ILC3 cells.

The work that I have performed on the function of PLZF and Zbtb1 for the differentiation of T-cells, and other immune cells, gave me the opportunity to start recently as Head of the T-cell facility in the ETIB. My goal was to change the focus of my research from basic to translational science. In the T-cell facility, I provide a double function: a) help investigators in the branch to perform experiments related to the analysis of immune end-points in clinical trials; and b) Implement and develop technologies for the discovery of novel immune therapeutics.

Damian Kovalovsky, Ph.D. (SS)
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The Clinical Corner

Section Editor: Alexandra Zimmer, M.D. (*SC*)

Getting to Know our Staff Clinicians

The main goal of this section is to increase the participation of Staff Clinicians, and make their work better known at NIH. In this issue, we interview our Staff Clinician, Stephanie L. Goff, M.D., to hear perspectives about her work and collaborations at NIH.

An Interview with Stephanie L Goff, M.D., FACS

What is your general role as Staff Clinician?

I have been a Staff Clinician in the Surgery Branch since 2014, returning as a fully-trained surgical oncologist to the clinics and labs in which I trained as a fellow (2005-2009) under the mentorship of Steven Rosenberg, M.D., Ph.D., James C. Yang, M.D., Richard M. Sherry, M.D., Marybeth Hughes, M.D., and Steven A. Feldman, Ph.D.. My most important role is the care of the patients that volunteer for our experimental protocols. That often starts with the review of charts of people curious about our trials and then moves to meeting the potential patients that travel to the Clinical Center to assess their eligibility and finally through enrollment onto one of our clinical trials. It calls upon not only my skills at the bedside and as a surgeon in the operating room, but also requires complex coordination in the translation of laboratory findings to highly individualized cell products. I am equally as passionate about educating the oncology community about our work through lectures and publications. That ranges from lectures for post-doctoral fellows to nursing in-services and education sessions to presentations at national and international meetings.

Could you point out steps and difficulties to implement a clinical trial?

While it sounds obvious, the first step is to really understand the question that you're asking and then frame it in a very specific way to make the result, positive or negative, have meaning. That kind of clarity leads to better trial design and eases the journey through the various regulatory mechanisms in place to protect our patients from undue risk. Once you have an approved protocol, it absolutely requires a coordinated team effort of referral nurses, research nurses and data managers to run a trial smoothly and maintain consistent patient accrual. That is an area in which I am incredibly fortunate. While I could describe many of the administrative burdens of running a trial as difficulties, I think the biggest challenge is less concrete and worthy of a wider discussion amongst researchers, regulators and patients. What is risk? What is undue risk? Should our definitions be different for patients with limited life expectancy? What level of risk is acceptable for early-phase, proof-of-principle, research?

What is your contact with Staff Scientists? Any report of cooperation from bench to bedside?

Under Dr. Rosenberg's leadership, the Surgery Branch has become a well-integrated translational research unit, building a unique cellular immunotherapy for each patient we treat. I couldn't possibly coordinate the portfolio of protocols we run without daily conversations with our staff scientists. The tumors I and our other surgeons send to the lab yield not only T-cells for therapy, but a treasure trove of information that informs the way we talk to our patients about the experimental possibilities we can offer them. In turn, after experimental treatment, we, as staff clinicians, can identify interesting clinical responses (both good and bad) for deeper study by our scientists. It's a cycle that continues to yield important information about cellular immunotherapy.

How do you see patient care at NIH? Can you give examples of benefits and limitations?

The supportive environment here at the "National Institute of Hope" takes away so many everyday stressors for our patients. They can focus on their families, their treatments, and their recovery without having to worry about insurance claims, benefits managers or pre-approvals, to name just a few of the many headaches patients have to contend with outside of Building 10. Our low patient:nurse ratios are the envy of those out in "the real world", and both our patients and our nurses have a more fulfilling care relationship as a consequence. No specialized center is without limitations, and there are likely places that deal with common problems better than we can here.

The Clinical Corner Con't

Section Editor: Alexandra Zimmer, M.D. (*SC*)

What is the career path of a Staff Clinician? Where do they go from here?

At one point, we were all first-year medical students with options stretching before us, thinking that when we decided on a residency we would have a path to follow into "adulthood." What I've found, particularly here at the NIH, is that the options never stop. While we all continue to care for patients, there are paths into leadership, into regulatory work, into editorial work, even options that can take one out of academia. Personally, I hope to continue to develop within the Surgery Branch and the Clinical Center.

Any final advice for new Staff Clinicians or about collaboration between Staff Clinicians and Staff Scientists?

My best advice is to remain engaged with the science behind the trials. The conversations I have with the staff scientists in our Branch are invaluable to my development as a tumor immunologist, and the more deeply I understand the hypotheses being tested, the more clearly I can present complicated data to our patients. Stay focused, involved, and immersed in the world of science available here. There is tremendous opportunity afforded by being surrounded by the world's experts. Take advantage of that.

Have you identified any differences or challenges in being a woman scientist? Any specific advice to other young women starting in that path?

This is always a tricky subject, as I have been incredibly fortunate to have been trained in environments where I was always encouraged to be a part of the conversation. In my current position, my ideas or suggestions are weighed by their merit and not by my gender, and I enjoy the respect and camaraderie of my colleagues. However, I know other women have had more significant difficulties. My advice is to remain assertive, and don't allow others to silence your voice. Develop a strong sense of self-awareness in order to identify and remedy your own limitations. Find a mentor (female or male) that sees your academic potential and wants to nurture it.

Stephanie L. Goff, M.D., FACS (SC)
Surgery Branch

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A Call for Content

We need your input! Send your articles or suggestions with subject title "The Dossier" to budhua@mail.nih.gov.

This newsletter is an avenue for you to express your ideas and thoughts on being a Staff Scientist or Staff Clinician at CCR and to make pertinent announcements.

Your contribution is very important to the success of The Dossier. Please send us your commentary, announcements and suggestions for topics/subject matter, and we will do our utmost to include your material in upcoming issues.

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