THE DOSSIER

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

June 2017 Issue 28

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 from Jonathan S. Wiest, Ph.D. about NCI's Office of Training and Education, and a special article by Andrew R. Byrd, Ph.D. The published work of Christophe Cataisson, Ph.D., is highlighted in our Author's Corner, while the SSSC Retreat Co-Chairs, Balamurgan Kuppusamy, Ph.D., and Siddhartha Datta, Ph.D., summarize the 2017 SSSC Retreat. Alexandra Zimmer, M.D., joins *The Dossier* as a Section Editor and summarizes our new Clinical Corner and Cynthia Masison, Ph.D., provides an important update on the SSSC Quadrennial Review. Jennifer C. Jones, M.D., Ph.D., discusses her collaboration with the Experimental Immunology and Transplantation Biology Flow Core and we highlight Evgeny Arons, 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)
Editor-in-Chief
Laboratory of Human Carcinogenesis

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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.

The Office of Training and Education

Numerous articles in this newsletter have highlighted the importance of the Staff Scientists and Staff Clinicians (SSSC) within the Intramural Research Program at CCR. The articles, by Senior Investigators and CCR Leadership, outline the notable SSSC contributions to science, publications, and mentoring our trainees. These are important and worthy contributions to CCR. However, not only are these staff positions highly important to the institution, they are also great careers. SSSCs often have the freedom, autonomy and independence to pursue projects of their own design. They can often apply for grants like the Director's Innovation Award, as well as other funding opportunities. I sometimes have Staff Scientist envy. Being able to mentor fellows, do experiments, analyze data and write papers is why I earned a PhD in the first place. I miss those activities as I sit in meetings and answer emails!

I also believe that a true appreciation of value is demonstrated not only by words, but by funding and other types of support. The proverbial, put your money where your mouth is! At NCI, there are several examples of institutional support for the SSSC population. The CCR Office of Training and Education (OTE) has long supported the group through assistance in building the SSSC organization. Working together with Ofelia Olivero, Ph.D., Drazen Zimonjic, Ph.D., Ana Robles, Ph.D., and others in 2004, CCR was among the first NIH intramural programs to establish such an organization. With the support of the OTE, the group began to identify the needs of the community and plan the first of many Annual Retreats and Fall Career Development Workshops. The SSSC Retreat has become an NCI-wide activity when CCR SSSC included their DCEG colleagues in the event. The OTE continues to support these activities with 13th Annual SSSC Retreat held in April of 2017. In addition, the OTE has also always included the SSSC community in courses and workshops hosted by OTE staff. Courses such as TRACO, Statistical Analysis of Research Data, Scientific Management Training and Preparing for Private Sector Careers have all been highly attended by the SSSC community. OTE also provides career counseling and help in transitioning to other career tracks.

The NCI also demonstrates its belief in the importance of supporting SSSC by being the first NIH Institute to support an extramural grant mechanism (Research Specialist, R50 mechanism) designed to fund this very type of position at universities across the country. Having highly trained, more senior research staff, supporting the science and mentoring in laboratories has been shown to create a more productive and stable research workforce. This mechanism also supports data scientists and core directors in a similar fashion as is done in the intramural program.

Overall, I believe the OTE, CCR, and the NCI as a whole, have demonstrated a strong commitment to the SSSC community. The SSSC are supported not only in words, but in action.

Jonathan S. Wiest, Ph.D.
Director, Center for Cancer Training
Chief, Office of Training and Education

Dr. Wiest is the Director of the Center for Cancer Training (CCT) and the Chief of the Office of Training and Education (OTE). Dr. Wiest came to the NCI in 2001 to establish the OTE as a resource for all non-tenured scientists in the Center for Cancer Research (CCR). In 2008, Jonathan was asked by the NCI Director to develop CCT.

The PI Corner

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

The Structural Biophysics Laboratory (SBL) focuses on understanding biological mechanisms through insights obtained from three-dimensional structures and the biophysics of intermolecular interactions. Our interests range from the ubiquitin regulatory pathway to kinase complexes and to RNA structural biology. The PIs in the SBL have a range of expertise and have come to this common ground via expertise in different structural methodologies. We have a very strong base in NMR spectroscopy, and we now have programs using x-ray diffraction crystallography (including the new X-ray Free Electron Laser (XFEL) at Stanford) and cryo-Electron Microscopy. Operating in a multi-disciplinary environment pursuing integrated structural biology places a high value on skilled team members, such as our Staff Scientists and our senior staff members. The SBL also houses two outstanding resources that are open to all CCR PIs: the Biophysics Resource (BR) and the Small Angle X-ray Scattering (SAXS) Core.

In order to conduct cutting edge research in area of interest of each PI and to provide support to CCR PIs in biophysics and SAXS, we rely on a fantastic group of staff scientists who provide critical expertise in specialty areas and interface with fellows and PIs throughout SBL and CCR. Xiang Chen, Ph.D. is an outstanding NMR spectroscopist working in the lab of Kylie Walters, Ph.D., Jason Stagno, Ph.D. specializes in micro- and nanocrystallography using the XFEL (Wang Lab), Olivier Soubias, Ph.D. is a membrane biophysicist with expertise in solid-state NMR (Byrd Lab), Lixin Fan, Ph.D. operates the SAXS core, and Sergey Tarasov, Ph.D. heads the Biophysics Resource. We also have critical support from Janusz Koscielniak, Ph.D. (NMR instrumentation) and Mr. Justus Benson (computer system administration). Each of these individuals brings unique skills to the research program and work together to make the SBL both a strong internal community and one that is open, cooperative, and collaborative with PIs and their groups throughout CCR. It is a unique quality to coordinate these skill sets to drive the mechanistic research in the SBL and the CCR. Perhaps more colleagues outside of the SBL are familiar with Sergey (BR) and Liz (SAXS), as they interact with tens of groups to provide cutting edge biophysical data that augments and supports the biological research throughout CCR. All of our staff scientists are very engaged in both the research and training environment, and they invigorate the research community. They are essential to all phases of our research program, from inception and planning of projects to conducting experiments and to presentation of results and representation of the SBL at scientific meetings. This level of independence and inter-reliability builds a strong team and provides an outstanding training environment for the next generation of scientists.

We welcome all our CCR colleagues to get to know this outstanding group of scientists and engage with us in broadening your own research perspectives.

Andrew R. Byrd, Ph.D.
Chief, Structural Biophysics Laboratory

Please share this newsletter with your colleagues and visit the SSSC website at sssc.nci.nih.gov.

The Quadrennial Review Corner

Changes to the Staff Scientist Quadrennial Review

CCR Staff Scientists are a diverse group of individuals making important contributions to their Lab/Branch as well as the overall mission of the NCI. Every four years a Staff Scientist/Staff Clinician undergoes a retrospective performance review: Quadrennial (Quad) Review. These reviews are required by the NIH and are an important tool used by CCR leadership for renewals, title changes, salary adjustments and to track personnel resources. This year the policy was amended to include review of Title 5/ Title 38 and Commissioned Corps as well as Title 42 employees to ensure consistency in performance evaluations of all staff. It is important to note, however, that for Title 5/ Title 38 and Commissioned Corps staff, the PMAP or COER will remain the evaluation that governs individual pay and promotion.

The process begins in September; the Staff Scientist is contacted to inform them that they are scheduled for review in the coming year. Their PI is also informed and a link to Quad Review forms is provided to facilitate preparation of the Quad Review Package. The Quad Review package consists of three parts. The first component is the recommending memo from the PI. The first part of the memo includes a checklist designed to identify the major duties of the staff scientist (SS) in their Lab/Branch. The second portion of the memo is a review of the staff scientist's performance *in the past 4 years* in the evaluation categories: **staff scientist's role; scientific productivity; presentations; participation in special interest groups-community involvement; mentoring/ teaching; tech transfer, involvement in GMP production, regulatory approval, CRADAs, INDs etc.; collaborations; continuing education; awards; significant achievements; and core activity and list of users/collaborators**. While there is no set format for the memo, most PIs use the given headings to be sure they have covered the relevant information. The information provided should focus on the achievements **in the past four years**. The PI can use this opportunity to explain any difficulties that may have occurred during the review period that had an impact on any of the areas of evaluation. The PI should provide specific details about the SS's involvement in the lab and the greater scientific community.

The second part of the Review package is the SS's CV. A standard CV template can be found at https://home.ccr.cancer.gov/intra/arc/documents/StaffScientistChecklistRenewal.pdf. Like the recommending memo, the CV should provide detailed information of the SS's career, particularly over the review period. The reviewers only have the information provided in the package to rate the SS so details such as the SS's role in collaborations and current positions held by their mentees will strengthen the package.

The final component of the Quad Review Package is a collection of at least two letters of recommendation from collaborators or scientists that know your work and can comment about your specific contributions in the past 4 years. The PI (not the SS) should solicit letters of support from individuals outside the Lab/ Branch and the NCI to indicate recognition by the broader scientific community.

Once completed, the package is submitted to the CCR ARC through the Lab/Branch Administrative Officer. Packages are submitted in December of the year prior to the review year. The package then comes to the Office of Scientific Programs. The packages are distributed to the Quad Review Panel that is comprised of the Promotion Review Panel and available CCR Deputies. Each package is assigned three independent reviewers that are responsible for rating the SS and presenting the package at the Quad Review Meeting. The Quad Review Meeting is held in March to discuss the packages and finalize the SS's ratings. We recognize that there are possible updates to a SS's package after submission to the ARC. Therefore, updates can be sent directly to our office for distribution to the reviewers. The SS and their PI should receive their Quad Review Report in early April.

Any SS and/or PI wishing to comment on the report can submit a response directly to me for inclusion in the final Quad Review Package that is submitted back to the CCR ARC and Senior Staff. While changes will not be made to the original rating, the response will be considered by CCR Leadership in making decisions on renewals and pay adjustments (funds permitting). The rating system is on a scale of 1-10: Outstanding (1-3), Outstanding-Excellent (3.1-3.5), Excellent-Outstanding (3.6-3.9), Excellent (4-6), Excellent-Good (6.1-6.5), Good-Excellent (6.6-6.9), Good (7), Good-Marginal (7.1-7.9), Marginal (8) and Unsatisfactory (>8). Senior Staff decided that, in addition to the descriptor, the actual number is now included in the report so that the SS would know where

The Quadrennial Review Corner Con't

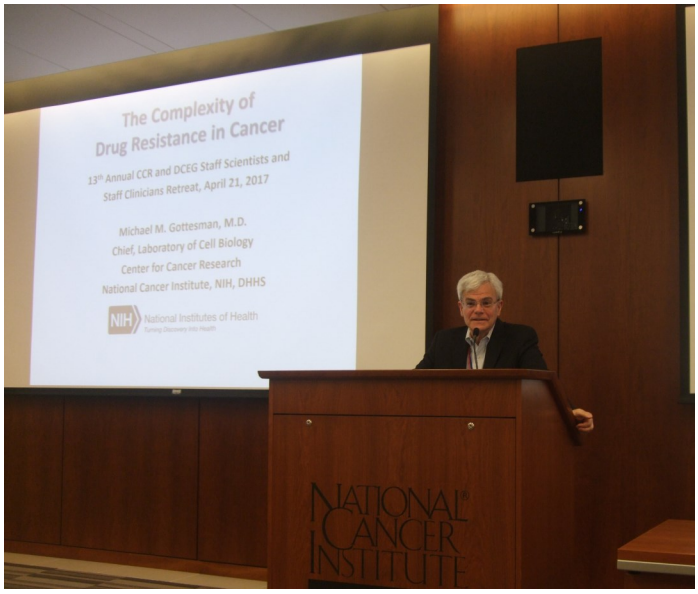
they rank in their category. This is most helpful for SS ranking Excellent or below.

The policy for SS renewal has also been changed from previous years with regards to a rating of "Excellent" or below. Specific details on the current renewal policy can be obtained from the CCR ARC https://home.ccr.cancer.gov/intra/arc/documents/StaffScientistChecklistRenewal.pdf.

This year 45 Staff Scientists underwent Quad Review. The group consisted of bench scientists, facility and core heads, bioinformatics specialists and administrators. This was the first Quad Review for ten of the SS. Four of the SS being reviewed were Associate Scientists. In the final rankings 80% of the SS received an outstanding in their descriptor. CCR Staff Scientists are an accomplished and diverse group of individuals making significant contributions to the intramural program.

Cynthia Masison, Ph.D.
Scientific Program Analyst
Office of the Director

The 2017 SSSC Retreat



Michael Gottesman, M.D., is pictured above delivering the keynote lecture at the 2017 SSSC Retreat.

The 13th annual Staff Scientists and Staff Clinicians (SSSC) Retreat, themed "Drug Resistance and Sensitivity", was held on April 21, 2017, at NCI-Shady Grove. The retreat, with over 110 participants from CCR, DCEG and Leidos, began with welcome remarks by Associate Staff Scientist, Nadya Tarasova, Ph.D. The morning session, moderated by Balamurugan Kuppusamy, Ph.D., began with a keynote talk by Michael Gottesman, M.D., Deputy Director for Intramural Research, NIH. His talk focused on our current understanding of the role of ABC transporters in various drug resistance mechanisms in cancer. This was followed by invited talks by four internationally-known scientists. Matthew Holderfield, Ph.D., (RAS drug discovery group team lead, Leidos) spoke about the strategies to target K-RAS, an oncogene mutated/overexpressed in many cancer types. Stephen Hughes, Ph.D., (Retroviral Replication Laboratory, CCR) highlighted the challenges in eradicating HIV in infected patients, and the contribution of long-lived infected clones to this issue. The application of the NCI-60 human tumor cell lines in preclinical screens for drugs in cancer, bacterial infection and thrombosis was described by Joel Morris, Ph.D., (Drug Synthesis

The 2017 SSSC Retreat Con't

Pictured from left to right are Matthew Holderfield, Ph.D., Stephen Hughes, Ph.D., Joel Morris, Ph.D., and Andre Nussenzweig, Ph.D.

and Chemistry Branch, DCTD). Andre Nussenzweig, Ph.D., (Laboratory of Genome Integrity, CCR) focused on genomic instability in therapy-associated AML. This session concluded with an interactive panel discussion, moderated by Anu Puri, Ph.D., involving the four invited experts.

Brown bag lunch sessions afforded an opportunity for informal discussions between the participants and invited speakers. In the afternoon, there were two poster sessions where SSSCs could present and discuss their research. As usual, these sessions allowed for a lot of interaction between SSSCs from diverse backgrounds, furthering opportunities for collaboration. A total of 58 posters were presented and judged by expert scientists. Following the poster sessions, there were five talks by SSSCs whose retreat abstracts had been judged to be exceptional, by a panel of specialists, including investigators from NCI. The five speakers were, Jennifer Kanakry, M.D., Brunilde Gril, Ph.D., Paul Boyer, Ph.D., Yanlin Yu, Ph.D., and Ngoc-Han Ha, Ph.D. Like the morning session, the afternoon presentations also covered many aspects of drug resistance mechanisms in various diseases, and was moderated by Yoshimi Greer, M.D., Ph.D., and Vladimir Majerciak, Ph.D.

This year, three travel awards as well as an outstanding mentor award were presented by Jonathan Wiest, Ph.D., Director, Center for Cancer Training, NCI. Two travel awards chosen from the poster presentations went to Noriko Sato, M.D., Ph.D., (Laboratory of Cellular Therapeutics, CCR) and Alberto Bartesaghi, Ph.D., (Laboratory of Cell Biology, CCR). This year a travel award for best oral presentation was introduced, and Ngoc-Han Ha, Ph.D., (Laboratory of Cancer Biology and Genetics, CCR) won the award for her outstanding presentation. The outstanding mentor award, which started last year, went to Brunilde Gril, Ph.D., (Women's Malignancies Branch, CCR) who was nominated by her interns and postdoc. Following the award ceremony, closing remarks were given by Doug Lowy M.D., Acting Director, NCI. He highlighted the importance of the SSSCs to the scientific progress in NCI and NIH and encouraged the SSSCs to continue in their efforts. Finally, in closing the retreat, participants got together for a group photograph.

Throughout the retreat there was an emphasis placed not only on the mechanisms underlying the drug resistance and sensitivity in cancer and other infectious diseases, but also on discussions about novel strategies to overcome this hurdle. The retreat brought together diverse researchers from the fields of cancer and other infectious diseases, and provided an opportunity to discuss recent developments. Many thanks to the attendees and especially the judges who made the trip to Shady Grove from Bethesda, Frederick, or wherever their workplace may be, for

The 2017 SSSC Retreat Con't

making this year's retreat a success! As always, we greatly appreciate the support of Jonathan Wiest, Ph.D., (Director, Center for Cancer Training and Office of Training and Education, NCI) as well the assistance from Angela Jones and Nicole Garner. This Retreat would not be possible without their invaluable support and help. We also thank Doug Nichols (NCI at Frederick Computer and Statistical Services specialist), who, set up an excellent website for us. This year's planning committee included Balamurugan Kuppusamy, Ph.D., Siddhartha Datta, Ph.D., Anu Puri, Ph.D., Sergey Tarasov, Ph.D., Abdul Waheed, Ph.D., Yoshimi Greer, M.D., Ph.D., and Vladimir Majerciak, Ph.D.

Before we end, we would like to emphasize the importance of participating in the annual SSSC retreat. This retreat is one of the few events conceived of, planned, and executed by you. It offers a wonderful opportunity to get to know your colleagues and their work, and opens opportunities for unexpected collaborations. See you at the retreat next year!

Sincerely,
Bala Kuppusamy, Ph.D. (*SS*)
& Siddhartha Datta, Ph.D., (*SS*)
Co-Chairs, 13th annual SSSC Retreat Committee

Please share this newsletter with your colleagues and visit the SSSC website at sssc.nci.nih.gov.

The Author's Corner

Section Editor: Cristina Bergamaschi, Ph.D. (*SS*)

MET Signaling in Keratinocytes Activates EGFR and Initiates Squamous Carcinogenesis

Cataisson C, Michalowski AM, Shibuya K, Ryscavage A, Klosterman M, Wright L, Dubois W, Liu F, Zhuang A, Rodrigues KB, Hoover S, Dwyer J, Simpson MR, Merlino G, Yuspa SH. Sci Signal. 2016 Jun 21;9(433).

Skin cancer is the most common form of cancer and typically develops in sunlight-exposed skin. The vast majority of skin cancers arise from basal or squamous cells, also known as keratinocytes, while melanoma develops from the pigment producing cells called melanocytes. Stuart H. Yuspa, M.D., co-chief of the Laboratory of Cancer Biology and Genetics, and his laboratory has been particularly interested in the early events in squamous cell carcinoma (SCC) development, the earliest change from a normal cell to a neoplastic cell. The laboratory had been studying the oncogene *Ras* for many years. *Ras* is a very prominent oncogene in human cancer, and one that we know will produce the benign tumor phenotype in our mouse skin model in the absence of any other mutation. So, a single mutation in *Ras* is sufficient to produce a benign tumor from an otherwise normal keratinocyte.

As a Staff Scientist in the Viral Pathogenesis Section of the Laboratory of Cancer Biology and Genetics, I had studied the facilitating role of inflammation on skin carcinogenesis using a transgenic mouse model called K5-PKC. I wanted to test whether keratinocytes-driven inflammation could also facilitate melanoma formation by crossing the K5-PKC mouse with a mouse model that was developed by Glenn Merlino, Ph.D, co-chief of the Laboratory of Cancer Biology and Genetics. This model overexpresses HGF (Hepatocyte Growth Factor), the ligand for MET, in all tissues, but particularly in the skin. Dr. Merlino's group observed that, in the MT-HGF model, melanocytes, which are normally located at the base of the hair follicle in pigmented mice, had relocated in upper part of the dermis because of HGF overexpression, so they resemble where melanocytes are present in normal human skin. Furthermore, they also observed in this model that, if when mice are exposed to ultraviolet light, they would get melanomas, but they also got a few squamous cell carcinomas of the epidermis. So we were particularly interested in understanding how cutaneous inflammation (driven by protein kinase C, PKC) would influence squamous cell tumors and melanoma development in double transgenic mice (DT or MT-HGF/K5-PKC). We treated DT mice first with a chemical carcinogen (DMBA or 7,12-dimethylbenzanthracene) known to produce tumors in skin, followed by multiple round of treatment with PKC activator TPA (12-O-tetradecanoyl-13-phorbol acetate). Surprisingly, DT mice develop fewer melanomas but exhibited greater sensitivity to squamous carcinogenesis. We also discovered that MT-HGF mice developed multiple tumors after treatment with TPA alone (without prior DMBA application). We showed that tumors resulting from carcinogen treatment contained activating mutations in *Hras* whereas none of the TPA-derived lesions showed *Hras* mutations. This result suggested that MET activation is sufficient to initiate skin SCC formation in a favorable environment, such as cutaneous inflammation driven by PKC. To test this idea further, we took advantage of the orthotopic grafting in vivo model that was developed in the laboratory where RAS-keratinocytes are implanted in a prepared graft site and form squamous papillomas in the absence of tumor promotion by TPA. We grafted HGF-overexpressing or WT skin cells on mice of the same genetic background, respectively. Encouragingly, only mice that overexpress HGF developed SCCs. In fact, transplantation of WT cells on HGF-expressing mice also formed squamous papillomas demonstrating that paracrine (host-derived) HGF can activate MET and substitute for the need of oncogenic RAS.

Next, we isolated primary keratinocytes from HGF-overexpressing mice and compared their behavior in culture to keratinocytes transduced with an oncogenic RAS. Both types of cells exhibited a spindle-like

shape and grew to a high density. Likewise, the cells showed similar activation of the epidermal growth factor receptor (EGFR) and the mitogen-activated protein kinase (MAPK) pathway, which lies downstream of EGFR. Inhibiting MET in HGF-overexpressing cells returned the cell shape to normal and reduced EGFR activity. Treatment with an EGFR inhibitor, however, had no effect on MET signaling, indicating a one-way interaction between the receptors.

We found that the HGF-induced activation of EGFR was due to increased expression of EGFR ligands. To be effective, the ligands must be released from the cell surface, and we showed that ligand release depended upon activation of the protease ADAM17. In turn, ADAM17 activation required HGF-mediated stimulation of the kinase SRC and induction of the proteins iRhom1 and iRhom2. To determine whether EGFR activation was necessary for MET-driven cancer, we grafted HGF-overexpressing cells on mice of the same genetic background, allowed tumors to form, and then treated the mice with the EGFR inhibitor gefitinib or a control. While the control had no effect, gefitinib treatment almost eliminated the established tumors, supporting the essential role of EGFR signaling (Figure 1).

Through a collaboration with Aleksandra M. Michalowski, Ph.D, Staff Scientist in Laboratory of Cancer Biology and Genetics, we could compare the global gene expression of activated *RAS* and HGF-overexpressing keratinocytes and found that their gene regulation was highly consistent. Using the top 372 consistent genes, we found activation of this *RAS*/MET signature in precancerous and cancerous human skin lesions, supporting the conclusion that MET signaling may occur early in many skin SCCs and persist through advanced stage. We also demonstrated that MET and HGF were abundantly expressed in human skin SCC tissue arrays. Together these data demonstrate that HGF and MET contribute to skin SCC formation via the activation of EGFR in a pathway parallel to that of oncogenic RAS.

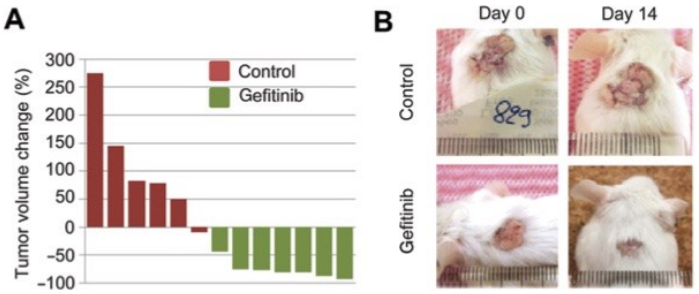


Figure 1. RAS-like phenotype and tumor development by HGF-overexpressing keratinocytes is mediated by EGFR. MT-HGF keratinocytes (6×10^6) were mixed respectively with 6×10^6 MT-HGF primary dermal fibroblasts before grafting in the interscapular region of syngeneic hosts. Once squamous papillomas were clearly established, mice were treated daily by oral gavage with vehicle control or gefitinib (100 mg/kg) for 2 weeks. (A) Waterfall plot of tumor response (% change of tumor volume) to 2-week treatment with gefitinib. Each bar represents tumor volume on an individual mouse. (B) Representative photographs of orthotopic squamous papillomas at the start of the treatment (day 0) and at termination (day 14).

Finally, this work provides mechanistic evidence that activated MET can initiate skin carcinogenesis. It is worth noting that *RAS* mutations are found in only 10-15% of human cutaneous SCCs but the EGFR-MAPK pathway is activated in most of them. We would like to pursue the MET-ADAM17 connection at the molecular level as it might uncover further therapeutic targets. We also hope that our report will foster the development of EGFR or MET topical inhibitors as the skin presents unique therapeutic opportunities.

Christophe Cataisson, Ph.D. (SS)
Viral Pathogenesis Section
Laboratory of Cancer Biology and Genetics

Christophe Cataisson, Ph.D., is a Staff Scientist in the In Viral Pathogenesis Section of the Laboratory of Cancer Biology and Genetics for the Center for Cancer Research. His role as a Staff Scientist includes: maintaining active collaborations within NCI and outside, leading studies on RAS mediated transformation of keratinocytes as well as research addressing the role of IL-17 during skin carcinogenesis. Additionally, he mentors post-baccalaureate fellows and provide guidance to postdoctoral fellows with various scientific aspects in the lab.

Introducing The Clinical Corner and Our New Section Editor

We are delighted to introduce the new "Clinical Corner" in *The Dossier*, which will appear in the upcoming September issue. Currently, most of the contributions by the SSSC to *The Dossier* are made by Staff Scientists concerning laboratory work. As a Staff Clinician, I believe we can report more on the clinical side of the CCR and, hopefully, add more Staff Clinician contributions to the SSSC newsletter.

In this new corner, we propose to discuss several different topics. This will include promising clinical trials that are open or in development at NIH. Many of these trials bring bench findings to the bedside, featuring a direct cooperation between Staff Scientists and Staff Clinicians. The current status of patient care will also be discussed along with how it can be improved at NIH. We would also like to discuss the gamut of Staff Clinician functions, their future goals and expectations. These topics will be expanded with your suggestions.

If you are interested in submitting an article for a future issue, please contact Alexandra Zimmer, M.D., (alexandra.zimmer@nih.gov). You are welcome to include any topics that may be related to this new section. We are looking forward to bringing this new section to *The Dossier* and hope that it will increase the visibility of our clinical research and hopefully, stimulate our Staff Clinician's participation and encourage new collaborations. Let us know if you are interested in contributing!

Alexandra Zimmer, M.D. (SC)
Women's Malignancies Branch

Dr. Alexandra Zimmer is a Staff Clinician in the Women's Malignancies Branch (WMB). Initially trained in Brazil, she completed her second Oncology fellowship at NCI/NIH in June 2015. During the fellowship, she worked in the laboratory of Patricia Steeg, Ph.D., and transitioned to work in the WMB, with the mission to translate laboratory work to clinical trials. Her main clinical and research interest is in breast cancer metastatic disease. More specifically, she is interested in developing translational clinical trials that target the study of metastasis prevention therapy and treatment of brain metastasis.

Please share this newsletter with your colleagues and visit the SSSC website at sssc.nci.nih.gov.

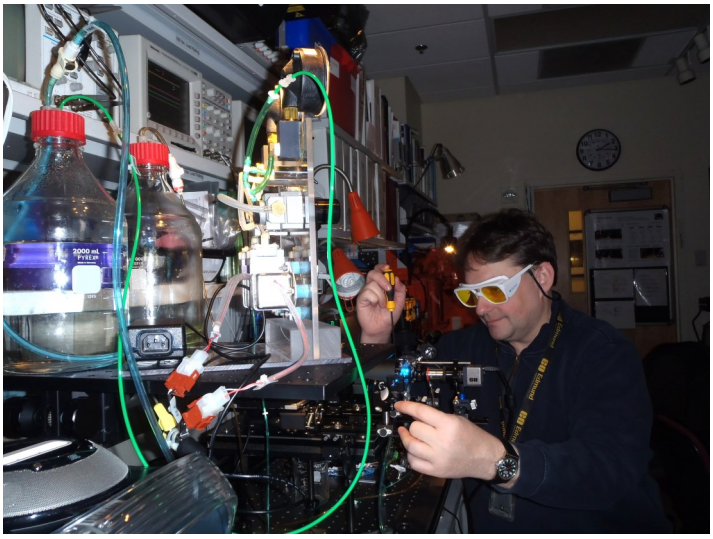
nanoFACS: Nanoscale Flow Cytometric Analysis and Sorting

Sensitive and specific biomarkers of disease status and treatment response are needed to optimize personalized and adaptive therapies. A major goal of my clinical practice in the Radiation Oncology Branch and basic research in the Vaccine Branch is to improve treatment selection and early- to mid-treatment adjustments for patients receiving radiation therapy or immune therapies to treat cancer. Exosomes (40-200 nm), microparticles (150-500nm), apoptotic bodies (>800 nm), and other submicron particles released by tumor cells and normal tissues into the circulation carry proteins, miRNA, and other molecules that can be used as biomarkers for monitoring treatment responses and customizing therapies.

To study the sizes, compositions, and functions of subsets of these extracellular vesicles (EVs), toward this goal of identifying better ways diagnose and monitor disease and responses to treatment, we developed nanoFACS, a cytometric method for single-particle analysis and sorting, which can be used to study individual extracellular vesicles or exosomes (*Scientific Reports*, May 2017), or sort viral vesicle subsets (*JCI Insight*, February 2017). Using these nanoFACS methods, along with a prototype next-generation analytical EV flow cytometer with single molecule detection capabilities, we are analyzing EV repertoires and EV subset cargo from patients during disease progression and during responses to treatment.

Our research has been supported throughout the course of these studies by several NCI Cores and Facilities and several scientists from those cores. Specifically, our work toward refining the technical capabilities of flow cytometers has benefited from the expertise of Bill Telford, Ph.D, who manages the NCI Experimental Immunology and Transplantation Branch (EITB) Flow Core, and whose expertise ranges from flow cytometer design and performance, to innovative laser methodologies and applications. In addition to Telford's contributions, Veena Kapoor, also provided substantial assistance with our research, especially several of our early studies that were performed on the Influx instrument in the NCI EITB Flow Core.

The foundation of our research efforts with exosome and small viral sorting and functional studies also received invaluable support and input from the Building 41 Vaccine Branch Flow Core manager, Kathy McKinnon, whose expertise in staining methods and protocol development were invaluable to our most recent publication that describes a new, optimized protocol for labeling exosomes for analysis and sorting with nanoFACS.



Bill Telford, adjusting a flow cytometer in the NCI Experimental Immunology and Transplantation Biology Flow Core.

In order to demonstrate the functional capabilities of labeled and/or sorted exosomes and viral vesicles (Morales-Kastresana et al, *Scientific Reports*, May 2017), we benefited from working with Tatiana Karpova, Ph.D, Head of the NCI/LRGBE Optical Microscopy Core, which specializes in high-end in vivo biophysical quantification by optical fluorescence microscopy, such as FRAP (Fluorescence Recovery After Photobleaching), FRET (Forster Resonance Energy Transfer), Single Molecule Tracking (SMT). Lisa Jenkins, Ph.D., Facility Manager for the Mass Spectrometry Resource within the Collaborative Protein Technology Resource, performed proteomic analyses of viral vesicle subsets sorted from plasma, and Brandon Keele, Ph.D., of the Frederick National Laboratory for Cancer Research Viral Sequence Analysis Core, analyzed the RNA sequences of the sorted vesicle subsets (Musich et al, *JCI Insight,* Feb 2017).

Overall, the results that we and our collaborators, Marjorie Robert-Guroff, Ph.D., and Jay Berzofsky,

The Core Corner Con't

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

M.D., Ph.D., achieved with these nanoFACS studies were made possible by the efforts, suggestions, and results from these Core Facilities and the outstanding scientists who lead and run those facilities. We hope that our results and methods will further help our team and others to study EV subsets with greater precision and focus, to determine the roles of EVs in the pathogenesis of cancer and tumor immune escape, and to develop new biomarkers for personalized and adaptive treatment strategies.

Jennifer C. Jones, M.D., Ph.D. (SC)
Molecular Immunogenetics &
Vaccine Research Section
Vaccine Branch

Jennifer Jones and Kathy McKinnon, performing nanoFACS together on the Astrios-EQ, in the NCI Vaccine Branch Flow Core.

The SSSC Corner

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

I received my PhD in immunogenetics after being trained in the laboratory of Rachel Ehrlich, Ph.D., at Tel Aviv University, Israel. During my graduate school years, I heard incredible things about the scientific work and network collaborations being done at NIH. When I first visited NIH in 2000, for the postdoc interview, I already knew that I wanted to stay here to build my career. I joined the laboratory of Robert J Kreitman, M.D., in the Clinical Immunotherapy Section of Laboratory of Molecular Biology at NCI, as a postdoctoral fellow in 2001, and here I am still at the same lab 16 years later. During my postdoctoral work, I focused on the characterization of clonal T-cell populations in classic Hairy Cell Leukemia (HCL). We found that increased CD3+/CD57+ T-cells may be a useful marker of abnormal TRBV repertoire in HCL patients, and should be considered in deciding whether patients should receive biologic antibody-based treatment.

As a Staff Scientist, I started new projects related to different aspects of HCL biology and immunogenetics. We study productive and unproductive V-D-J rearrangement, somatic hypermutation pattern and CDR3 region in HCL and HCLv derived immunoglobulin heavy variable heavy chain genes (IGHV). The analysis of the immunoglobulin genes in chronic lymphocytic malignancies contributes significantly toward understanding the molecular pathogenesis of the diseases, as well as the assignment of the malignant B cells to their normal counterpart. Bias in the usage of certain immunoglobulin (IG) heavy variable (IGHV) genes brings evidence for the contribution of antigens and/or superantigens in lymphomagenesis by stimulating the proliferation of malignant cells with distinctive IG receptors. Somatic hypermutation (SHM) of IG variable genes after antigen stimulation

The SSSC Corner Con't

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

occurs in the germinal centers (GC) through T-cell dependent manner or T-cell independent mechanism. The mutation status of IGHV genes has been established as one the important molecular markers in defining prognostic groups in some types of B-cell leukemias. The unique sequence of IGHV CDR3 region can be used as a leukemia marker and for very sensitive patient MRD assays.

When I am not working, I love to read books and listen to music. I like to run and swim. My family loves to travel. Our favorite destination in the US is the Southwest, where we have explored many national parks and canyons.

Evgeny Arons, Ph.D. (SS)
Clinical Immunotherapy Section
Laboratory of Molecular Biology

Evgeny Arons with his daughters Danielle and Ann at Bryce Canyon.

Announcements

Congratulations!

2017 SSSC Retreat Award Winners

Noriko Sato, M.D., Ph.D., (Poster presentation), Laboratory of Cellular Therapeutics

Alberto Bartesaghi, Ph.D., (Poster presentation), Laboratory of Cell Biology

Ngoc-Han Ha, Ph.D., (Oral presentation), Laboratory of Cancer Biology and Genetics

Brunilde Gril, Ph.D., (Oustanding Mentor), Women's Malignancies Branch

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