THE DOSSIER

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

September 2016 Issue 25

From the Editor

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

This issue contains important messages from the Director's Office, by Tom Misteli, Ph.D., and and Jason Levine, M.D., and a special article by Susan Mackem, M.D., Ph.D. Updates on the SSSC Professional Development Committee and information about the upcoming SSSC Professional Development Day is provided by Swati Choksi, Ph.D., We feature Duane H. Hamilton, Ph.D., in the SSSC Corner, while the published work of Krista Delviks-Frankenberry, Ph.D., is highlighted in our Author's Corner. Tristan Sissung, Ph.D., M.S., shares his collaborative work with The Genomics Laboratory and we also introduce our new Conferences Corner, which will be overseen by our new Section Editor, Majda Haznadar, Ph.D.

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

In This Issue

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From the Office of the Director

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

The CCR has nearly 2,500 staff members, more than 230 investigators, and an amazingly diverse scientific portfolio. With it comes equally diverse scientific IT needs. We are fortunate to have the CCR Office of Information Technology (OIT) to support scientists and clinicians with these specialized needs.

As many know, the Center for Biomedical Informatics and Information Technology (CBIIT) is NCI's organization responsible for general IT support — CBIIT manages the wires behind your walls, the computers on your desktops, and many of the other general resources you use. When your needs turn more scientific though, it is CCR's OIT that can serve as a resource to help CCR staff identify how best to solve specific computational problems and continue driving science forward. OIT runs systems to track biospecimens, assist with the collection and management of research data, guide protocols through approval, handle the referral of outside patients into studies, and provide centralized documentation and tracking for research projects. OIT also collaborates with PIs and their teams to address other research needs as they arise, from data storage and high-performance computing to digital microscopy and administration of scientific systems.

OIT also manages Labmatrix, a full research information management system. This advanced tool allows users to model, store, and retrieve data about all phases of a research project. Whether you work in a basic science lab or at the bedside, OIT will work with you to understand what data you want to collect, how you want to collect it, what kinds of questions you want to be able to ask of it, and then use Labmatrix to implement the structure and forms that allow you to do so. Labmatrix is entirely web-based, so you can use it from any computer that is on the NIH network. It is also access-controlled, meaning that only individual research groups have access to their data, and that access can be further refined in ways, such as limiting the visibility of protected health information, or giving view-only access. And it is extensible, meaning that we can provide bridges between Labmatrix and other NCI or NIH systems so that your data can be combined, routed, and analyzed however you see fit. OIT has already built bridges that bring clinical and demographic data into Labmatrix, and Labmatrix is the first research system that can receive orders from the Clinical Center's medical record and send results back into the record.

OIT also works closely with other NCI and NIH computational resources. As CBIIT implements government-wide IT mandates within NCI, OIT helps refine those plans to recognize the unique needs posed by CCR's intramural scientific activities, and helps find solutions to challenges that arise along the way. OIT will also be a valuable partner for the newly-announced CCR Cancer Data Science Laboratory (CDSL), which will significantly strengthen our bioinformatics capacity by creating a dedicated computational research program and provide a high-level collaborative platform for researchers to tackle data-intense problems in cancer research.

OIT is here to help you! The goal is for investigators to never find themselves caught between a worthy scientific goal and an IT requirement — with the right tools and processes, we can (almost!) always find a way. Please don't hesitate to reach out to OIT with your scientific IT needs, and we'll do our best to get you and your science moving forward.

Tom Misteli, Ph.D.
CCR Director

Jason Levine, M.D.
Associate Director for IT & Clinical Informatics, CCR

The SSSC Professional Development Committee

The SSSC Professional Development Committee was started in 2010. This committee was formed to address the needs of the SSSC community in terms of continuing education and issues related to the professional development and progress of our community. To this end, the committee, under the leadership of Christophe Marchand, Ph.D., has tackled many issues over the years. Recently, Christophe has taken on a new position as a Health Scientist Administrator for the Center for Research Strategy. The SSSC Professional Development Committee plans to continue to provide the SSSC community with professional development opportunities. We wish Christophe all the best in his new position. In the past, we have taken on several projects including creating the SSSC Alumni database, updating the SSSC Handbook, streamlining the Quad Review process, offering information on SSSC displacement issues and providing training through the SSSC Professional Development Day. With the recent leadership change at the NCI, it is important, now more than ever, for us to be aware of changes that pertain to us and therefore, as a committee, we plan to tackle issues that are most relevant to the SSSC. If you see an issue, problem or learning opportunity, do not hesitate to bring it to the attention of the Professional Development Committee. In fact, join us!! We are always looking for people that want to work for the betterment of the SSSC community.

Swati Choksi, Ph.D. (SS)
Laboratory of Genitourinary Cancer Pathogenesis

The Fourth Biennial NCI SSSC Professional Development Day

The SSSC Professional Development Committee is pleased to announce the Fourth Biennial NCI SSSC Professional Development Day to be held in the **Porter Neuroscience Center (Building 35 Room 610)** on **September 23, 2016**. This year's program will tentatively cover the following topics:

1) Technology Transfer - What you need to know as a Staff Scientist about patents, invention reports and CRADAs
2) USA Jobs - Inside tips on preparing a successful USA Jobs application
3) We Want You! - NIH jobs for those ready to leave the bench
4) Quadrennial Review - How you can be outstanding in your next Quad Review

Please join us for this unique professional development opportunity. Please register by September 16, 2016 at: https://ncifrederick.cancer.gov/events/BiennialSSSC2016/register.asp

The PI Corner

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

In a changing research environment with smaller labs and more limited resources, maintaining productivity and doing cutting edge research poses a bigger challenge than ever. In such a climate, the Staff Scientist has come to play an integral and critical role and is expected to function as far more than a 'super postdoc'. I've recently joined the CCR Promotion Review Panel (PRP), which assesses Staff Scientist productivity in quadrennial (quad) reviews, and have noticed that although the roles and expectations for staff scientists heading scientific core facilities are usually clearly defined, this is often not the case for 'bench' Staff Scientists who engage mainly in their own research projects. Primary research Staff Scientists and their PI supervisors may not always be well informed and have misconceptions about what's expected. Here, I'm offering my advice from recent experience with the PRP thus far (which may not necessarily reflect the views of all other PRP members).

The Staff Scientist position offers great opportunities to contribute to science and also comes with substantial responsibilities. Yes – research productivity, both in terms of primary publications and reviews and presentations at national and international conferences, is expected and is essential. Presenting your work at major conferences is an important component of this, which Staff Scientists may neglect given their busy schedules and responsibilities – DON'T. Presenting your research at meetings is important to publicize your work, stay up to date with unpublished, breaking results your field, and network and collaborate with colleagues. It will keep you sharp and engaged and shouldn't be perceived as optional. Interaction and collaboration with the broader scientific community at NIH is likewise an important component of scientific success and is beneficial in moving forward your research as well as achieving a successful quad review. For example, organizing and participating in local meetings and seminar series is a great way to keep up contact and interact with other intramural scientists who have related interests and potential expertise to offer, and who may benefit from your expertise as well.

Training new fellows and actively contributing to their mentoring process is another important Staff Scientist role, especially in small labs, where maintaining an institutional memory of scientific expertise can often be more difficult. Another component of this role is keeping abreast with new and changing technologies and developments in your field of research. You're both contributing to the success of the next generation of researchers and ensuring the future productivity of your lab. Attending major conferences affords one way to stay current; attending courses and workshops (eg. CSH, EMBL) to learn new technologies that will benefit your research and your lab is another area that should be pursued. Both of these activities will sustain and advance the collective scientific expertise of the lab and ensure continued great science for the future. Following these guidelines will be a sound investment both for achieving successful quad reviews and most importantly, a productive and rewarding career that 'pushes the envelope' of biomedical research.

Susan Mackem, M.D., Ph.D.
Senior Investigator, Cancer and Developmental Biology Laboratory

Introducing The Conferences Corner and Our New Section Editor

We are delighted to introduce the new "Conferences Corner" in *The Dossier*, which will appear in the upcoming December issue. This section will feature summaries of national and international meetings our Staff Scientists and Staff Clinicians attend. In this manner, we hope to be able to provide highlights from meetings many of us would like to attend, but are unable to due to lab constraints, budget restrictions or other prior obligations. Herein, we would like to call for content for the upcoming issue. If you will be attending any Keystone, Gordon or AACR-based conferences, please consider contributing to the Conferences Corner. If you are interested in submitting an article for the December or a future issue, please contact Majda Haznadar, Ph.D., (haznadarm@mail.nih.gov). You are welcome to include any conference highlights or impressions that you think would be of interest to our readers, including, but not limited to, late breaking research, emerging themes, interesting speakers and/or topics, conference setting, etc. You may also send a photo from the conference that would complement the article, for example of yourself at your poster or oral presentation.

We are looking forward to bringing a new section that will allow you to receive witness-accounts of conferences of interest that you were unable to attend. Let us know if you are interested in contributing!

Majda Haznadar, Ph.D. (SS)
Laboratory of Human Carcinogenesis

Dr. Majda Haznadar is a Staff Scientist in Dr. Curt Harris' Laboratory of Human Carcinogenesis. Her research interests include utilization of mass-spectrometry based metabolomics approaches for the identification of non-invasively measured risk, early diagnostic and prognostic biomarkers in lung cancer. She has also developed an independent project in the lab, wherein she is investigating a role of vitamin D in anti-tumorigenic processes in lung cancer. She has mentored several fellows in the lab, and continues to enjoy transferring knowledge and working with many exceptional colleagues. Dr. Haznadar is also involved in several collaborations within and outside of NCI.

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*)

Minimal Contribution of APOBEC3-Induced G-to-A Hypermutation to HIV-1 Recombination and Genetic Variation

Krista A. Delviks-Frankenberry, Olga A. Nikolaitchik, Ryan C. Burdick, Robert J. Gorelick, Brandon F. Keele, Wei-Shau Hu, Vinay K. Pathak. PLoS Pathog. 2016 May 17;12(5): e1005646

High levels of genetic variation and diversity exist within the circulating human immunodeficiency virus type 1 (HIV-1) population. Production of ~10^{11} HIV-1 virions per day/patient leading to ~10^{9} infected target cells (1) generates a large population of variants with great evolutionary potential. This diversity further allows HIV-1 to escape immunological and pharmacological selection pressures, thwarting current treatment and vaccine efforts. Therefore, in order to move forward in the development of effective treatments against HIV-1, it is important to understand all sources of HIV-1 genetic diversity.

For HIV-1, as well as other retroviruses, one major source of genetic diversity is error-prone viral replication. Virally encoded reverse transcriptase enzyme (RT) converts the ssRNA HIV-1 genome into dsDNA during a process known as reverse transcription. The enzyme error rate has been previously measured to be between 1.4-3.4 × 10^{-5} mutations/bp/replication cycle (1). Furthermore, since HIV-1 integrates its genome into the host chromosome to form a provirus, contributions to genetic diversity can also arise from host DNA polymerase through normal cell division. However, the contribution to viral genetic variation from this mechanism is considered negligible. In contrast, genetic variation can arise through errors during RNA polymerase II-mediated transcription, and its contribution to HIV-1 genetic variation has not been determined. The third major source of genetic diversity comes from the inherent property of retroviruses in that HIV-1 packages two copies of its genome into every particle. During reverse transcription, RT can also switch templates between the two copackaged genomes, reassorting mutations through recombination. It has been measured that HIV-1 will undergo ~9 templates switches/genome/replication cycle (1).

Interestingly in 2012, it was discovered that a host protein expressed during viral infection, known as apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G), was able to block HIV-1 infection in the absence of one of the virally-encoded proteins, Vif (virion infectivity factor). The family of APOBEC3 proteins largely inhibit HIV-1Δ*vif* by inducing cytidine deamination during reverse transcription, leading to extensive guanine to adenine (G-to-A) mutations in the HIV-1 genome, known as hypermutation, which lead to defective and replication incompetent HIV-1. HIV-1 has cleverly evolved to evade APOBEC3 proteins by expressing Vif, which binds and targets APOBEC3 proteins for proteasomal degradation. However, analysis of patient HIV-1 sequences shows that Vif is not always successful at degrading APOBEC3 proteins.

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

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

The role of hypermutation in HIV-1 genetic diversity is not clear. One side of the debate proposes that sub-lethal doses of G-to-A mutations can contribute to genetic variation and drug resistance, whereas others propose that G-to-A hypermutation is an "all or nothing " phenomena (1). A collaborative effort from the Viral Mutation Section directed by Vinay K. Pathak, Ph.D., and the Viral Recombination Section directed by Wei-Shau Hu, Ph.D., within the HIV Dynamics and Replication Program, led to a recent publication in *PLoS Pathogens,* dissecting whether APOBEC3-induced hypermutation was contributing to genetic variation in the replicating HIV-1 population of infected patients.

Krista Delviks-Frankenberry, Ph.D., Staff Scientist in the Viral Mutation Section, together with co-authors, measured the contribution of hypermutation to HIV-1 recombination and genetic variation by analyzing both *in vitro* experiments and *in vivo* patient data. Cell lines were established that were dually infected with a wild-type HIV-1 genome and a HIV-1 genome containing either high or low levels of hypermutation. Each provirus also contained an inactivated GFP gene at either the 5' or 3' end; thus homozygous particles would never express functional GFP. However, for heterozygous virions, in which a wild-type and a hypermutated genome were copackaged together, recombination could result in the expression of functional GFP. This assay has the ability to measure in a single cycle the frequency of recombination between a hypermutated and wildtype genome, by analyzing recombinants only from infected target cells that fluoresced green. The results showed that even though the presence of hypermutation reduced the homology between the copackaged RNAs, no significant difference in the recombination frequency was found, as the same numbers of crossovers were observed in the presence or absence of hypermutation. Thus hypermutation was shown not to affect the rate of recombination (Figure 1, X^a).

Another consequence of hypermutation is lethal mutagenesis or sublethal mutagenesis. Lethal mutagenesis generates dead-end proviruses that cannot increase genetic variation on their own. However, if the same cell is infected with a replication-competent virus, then portions of the hypermutated provirus could be rescued during copackaging and recombination. This is also true for sublethally hypermutated proviruses. Analysis of 413 recombinants showed only 5 of the recombinants contained G-to-A hypermutations without stop codons leading to a contribution of hypermutation to the overall HIV-1 mutation rate of 3.9×10^{-5}/bp/replication cycle. This is within 2 -fold of the measured HIV-1 mutation rate/bp/replication cycle. Furthermore, since the frequency of copackaging a hypermutated and wildtype genome is extremely rare in HIV-1 infected patients (<1%), the data supports that the contribution of genetic variation from hypermutation to the HIV-1 mutation rate is substantially lower than that of error-prone viral replication (Fig. 1, X^b, X^c).

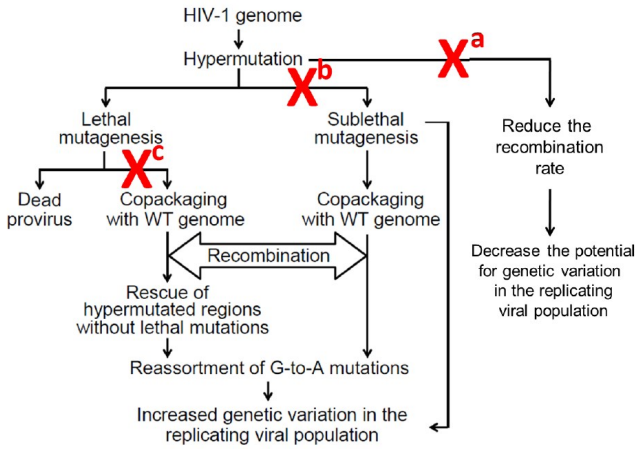

Figure 1. Flow chart showing potential contributions from G-to-A hypermutation to HIV-1 genetic diversity. The contribution of hypermutation to HIV -1 genetic diversity was found to be negligible (red Xs).

To support the findings even further, HIV-1 patient-derived hypermutated sequences were analyzed to determine the frequency of sublethal mutagenesis. Patient sequences were found to carry on average 268 G-to-A mutations which resulted in 47 stop codons for sequences predominantly hypermutated by APOBEC family member A3G, giving the probability of generating a sublethally hypermutated provirus without any stop codons on the order of 4×10^{-21}. *In silico* modeling also showed that an HIV-1 genome having 10-15 G-to-A mutations would have the greatest impact on HIV-1 diversity (5-6-fold higher than error-prone replication). However, at the observed frequency of G-to-A mutations (268/genome), the contribution of APOBEC3G to viral genetic variation through recombination is far less than that of error-prone replication. In summary, the data supports that G-to-A hypermutation does not significantly contrib-

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

-ute to HIV-1 genetic variation. Analysis of hypermutated sequences from infected patients indicates that the frequency of sublethal mutagenesis is negligible and its contribution to viral variation is substantially lower than that of error-prone viral replication.

Krista Delviks-Frankenberry, Ph.D. (SS)
Viral Mutation Section
HIV Dynamics and Replication Program

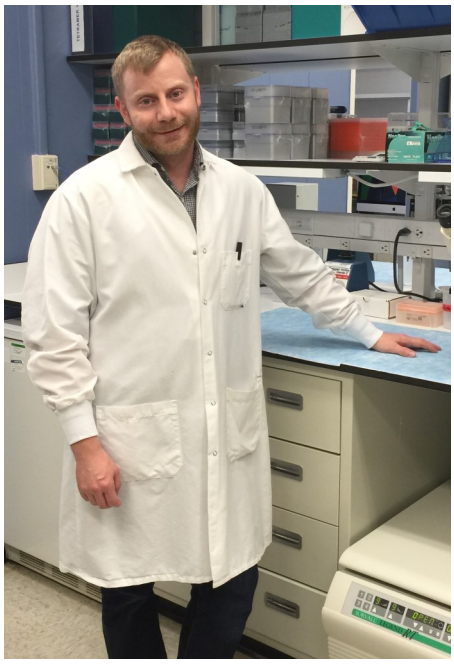
Dr. Krista Delviks-Frankenberry is a Staff Scientist in the Viral Mutation Section of the HIV Dynamics and Replication Program at NCI at Frederick. Her role as Staff Scientist includes supporting and carrying out research projects in Dr. Pathak's lab, providing training to summer students and post-docs, and developing new tools like TALENs and CRISPRs to implement in the lab. Her studies have made major contributions to the understanding of HIV-1 drug resistance and the origins of Xenotropic murine leukemia virus-related virus (XMRV).

References:

1. References cited within PLoS Pathog. 2016 May 17;12(5):e1005646.

The SSSC Corner

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



My first exposure to the amazing breadth of research being performed at the NCI came as I interviewed for a position as a Visiting Fellow in the Laboratory of Tumor Immunology and Biology (LTIB). Coming from a small lab which focused on the activation requirements of naïve CD4+ T cells, the LTIB was my first exposure to true translational research. Even after more than eight years, I continue to be amazed at the speed at which researchers and clinicians at the NCI are able to move novel treatment modalities from pre-clinical observations into clinical studies.

During my time as a post-doctoral fellow in the LTIB, I was very fortunate to work with the mentorship of two amazing scientists, Jeffrey Schlom, Ph.D. and Claudia M. Palena, Ph.D. Together, we examined the development of therapeutic resistance by carcinoma cells. In particular, we examined how the acquistion of mesenchymal-like features associate with increased resistance to cytotoxic therapies, including immunotherapy. In collaboration with researchers at the National Center for Advancing Translational Sciences (NCATS), we were able to perform a high-throughput screen to identify, and re-purpose an FDA-approved compound which mitigates the increased resistance of mesenchymal-like lung carcinoma cell lines, rendering them more susceptible to immune-mediated killing.

As a newly appointed Staff Scientist in the LTIB, I have returned to my immunological roots. I am currently evaluating techniques to identify antigens uniquely expressed by the patient's tumor. It is our belief, that combining these patient-specific vaccines,

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

With our current panel of 'off-the-shelf' cancer vaccines will improve the breath of the anti-tumor immunity generated, resulting in greater immunological control of tumor growth.

Outside of the lab, I am a voracious napper, and enjoy spending my free time reading, exercising and spending time with friends. Arguably, none of my hobbies lend themselves very well to photographs, so I have included one of me being terrorized by a clown last halloween…

Duane H. Hamilton, Ph.D. (SS)
Laboratory of Tumor Immunology and Biology

Duane is pictured above meeting the world's scariest clown during a Halloween street fair in Fort Lauderdale.

Announcements

Communication is important! Follow CCR on Twitter @NCIResearchCtr. Don't forget to send all of your news to us at tellccr@mail.nih.gov. We want to hear about awards, honors, accepted papers, Grand Rounds speaker suggestions, and possible nominees for scientific awards.

Attend!

The Fourth Biennial NCI SSSC Professional Development Day

Porter Neuroscience Center (Building 35 Room 610) on September 23, 2016

Register by September 16, 2016: https://ncifrederick.cancer.gov/events/BiennialSSSC2016/register.asp

The Core Corner

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

Pharmacogenomics at NIH

The Genomics Laboratory, Cancer Research Technology Program (Frederick), is collaborating with the Clinical Pharmacology Program, the Pharmacy Department, and several other NIH groups to launch pharmacogenomics efforts for the purposes of research and patient treatment. This collaboration has led to several discoveries of novel pharmacogenomic markers that are important in therapeutic outcome, and has been useful to prevent undue toxicity in patients who are receiving drugs with known pharmacogenomic associations.

Using an Affymetrix Drug Metabolizing Enzymes and Transporters (DMET) array, which tests 1936 pharmacogenomic variants in 235 genes that regulate drug absorption, distribution, metabolism, elimination, and activation (ADMEA), we have conducted several retrospective studies on patients with unusual toxicity or pharmacokinetics to determine whether pharmacogenomic variants are responsible for these outcomes. For instance, we found that patients with genotypes that predict faster metabolism of an anticancer agent, belinostat, appear to require greater doses, whereas those with slower metabolism variants require a lower dose and are more susceptible to belinostat-related toxicities. This and other discoveries have recently been leveraged into prospective clinical trials with *a priori* CLIA assessment of pharmacogenes in the enrollment criteria. Such assessments are used to either determine whether patients are eligible for therapy or if patients will receive high or low doses of certain agents. We anticipate that these clinical trials will lead to more accurate dosing guidelines in early clinical trials, in which safety and pharmacokinetics are primary endpoints.

In the hospital setting, collaborations were initiated between several stakeholders at the NIH Clinical Center to implement pharmacogenomics testing in drugs that have specific dosing guidelines recommended by the Clinical Pharmacogenomics Implementation Consortium (CPIC). Since the DMET array is useful to test these ADMEA genes, we have also used this technology to ascertain genotypes and have included pharmacogenomics reporting into CRIS. Due to these efforts, the NIH has become a pioneer in offering greater precision in patient treatment.

Tristan Sissung, Ph.D. , M.S. (SS)
Clinical Pharmacology Program
Office of the Clinical Director

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

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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