

**NATIONAL CANCER INSTITUTE AT FREDERICK (NCI@F)**  
**INSTITUTIONAL BIOSAFETY COMMITTEE**  
**MINUTES**  
**JANUARY 19, 2016**

**CALL TO ORDER / ANNOUNCEMENTS**

The NCI at Frederick Institutional Biosafety Committee was convened at 12:00 pm in Building 549 Executive Board Room with the following members in attendance:

Voting (Quorum = 8)

- |  |  |
|--|--|
| <input checked="" type="checkbox"/> Michael Baseler              | <input checked="" type="checkbox"/> Sarah Hooper           |
| <input checked="" type="checkbox"/> Theresa Bell                 | <input checked="" type="checkbox"/> Serguei Kozlov         |
| <input checked="" type="checkbox"/> Rev. David Betzner           | <input type="checkbox"/> Dan McVicar ( <i>regrets</i> )    |
| <input checked="" type="checkbox"/> Stephen Creekmore            | <input checked="" type="checkbox"/> Bradley St. Croix      |
| <input type="checkbox"/> Eric Freed ( <i>regrets</i> )           | <input type="checkbox"/> Lucien Winegar ( <i>regrets</i> ) |
| <input type="checkbox"/> Melinda Hollingshead ( <i>regrets</i> ) | <input checked="" type="checkbox"/> Sharon Altmann         |
| <input type="checkbox"/> Stephen Hughes ( <i>regrets</i> )       | <input checked="" type="checkbox"/> Robin Sun              |
| <input checked="" type="checkbox"/> Antonio Valentin             | <input checked="" type="checkbox"/> Jatinder Gulani        |

Non-Voting

- Walter Hubert
- Karen Barber

Visitors

Ted Witte  
Sam Denny

**APPROVAL OF MINUTES FROM THE DECEMBER 2015 MEETING**

The minutes from the December 15, 2015 meeting were approved with minor clarifications. A motion to approve and a second were made. (For: 11; Against: 0; Abstain: 0 )

**ACCIDENT REVIEWS :**

Employee working in building 571 was performing primary lymphocytes from Black 6 into RAG KO mice. This is work with retroviral vectors. The employee completed her experiment and was cleaning the scissors. When she was wiping the clean scissor to dry, that is when she stuck her left middle finger with the scissors.

**REVIEW OF PROTOCOLS**

***NEW REGISTRATIONS***

- None presented.

***RENEWAL REGISTRATIONS***

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- ❖ Andre Nussenzweig – 15-60 (12-63): Expression of MLL fusion proteins in BM cells for transplantation to assess tumor formation in vivo. Our lab is studying the role of DNA repair response genes in tumorigenesis. We believe that deletion or inhibition of proteins in the DNA repair response pathways can impair or reverse MLL fusion induced acute myeloid leukemia (AML). To test this in vivo we will 1) transfect HEK293T cells to produce murine ecotropic retrovirus carrying DNA encoding a murine MLL fusion protein, 2) infect wildtype or knockout mice BM cells to stably express the MLL fusion protein, 3) IV inject these transformed cells into recipient mice, either C57BL6 or NRG, 4) follow progression of leukemia to up to 140 days. A motion to defer to February 2016 with the request to resubmit the registration.  
(For: 11; Against: 0; Abstain: 0)
  
- ❖ Vinay Pathak – 15-62 (07-37): Mechanisms of retroviral replication. We seek to understand how retroviruses replicate and how they evolve to acquire resistance to antiviral drugs and human antiviral defense proteins called restriction factors. We are studying how APOBEC3 proteins, a family of host cytidine deaminases, inhibit HIV-1 replication, and how the viral Vif protein overcomes these host defenses. We are screening for small molecule inhibitors of APOBEC3-Vif interactions, elucidating the structure and function of APOBEC3 proteins, and identifying host genes that facilitate the Vif-APOBEC3 interactions. We are exploring mechanisms of antiviral drug resistance and retroviral replication. We are also using APOBEC3 proteins tagged with fluorescent proteins such as yellow fluorescent protein to label HIV-1 virions that can complete one cycle of replication but have defects in many essential genes such as envelope and/or reverse transcriptase, and accessory genes such as Vif, Vpr, Vpu and Nef. A motion to defer this registration was made, pending clarifications and an observation, by Steve Creekmore and seconded by Serguei Kozlov,.  
(For: 11; Against: 0; Abstain: 0)
  
- ❖ Lisa Ridnour – 15-63 (12-77): Role of Nitric Oxide in Breast Cancer Disease Progression. Breast cancer is a heterogeneous disease defined by distinct tumor phenotypes that vary in prognosis and response to therapeutic agents and remains the second leading cause of cancer related deaths among women in the United States. Although standard therapeutics have improved the outlook and quality of life, 16% of women with regional lesions and 76% of women with metastatic disease continue to lose their fight against breast cancer within the first five years of diagnosis. Clinical management of breast cancer currently employs diagnostic patient evaluations aimed at designing more personalized therapeutic modalities including tamoxifen for the treatment of estrogen receptor positive (ER+) disease, or trastuzumab for human epidermal growth factor receptor-2 (HER-2) positive disease. However, further identification of biomarkers for improved therapeutic response is warranted. Toward this end, inflammation is clearly important in carcinogenesis and cancer progression. Under normal inflammatory conditions, inflammation is resolved by feedback mechanisms. When these feedback mechanisms are dysregulated, as it occurs in inflammatory diseases and cancer, chronic inflammation ensues. In cancer, this process is often referred to as “the wound that does not heal”. Nitric oxide (NO) derived from the inducible nitric oxide synthase (iNOS or NOS2) enzyme is a key inflammatory mediator. Importantly, NOS2 is up regulated in

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inflammatory diseases that pose an increased risk for cancer development, and predicts poor disease-specific survival when elevated in tumors of several types, including breast cancer. We hypothesize that NOS2-derived NO perpetuates feed-forward inflammatory signaling loops that promote tumor progression to more aggressive metastatic and drug-resistant phenotypes. A goal of our group pertains to the elucidation of this pro-tumorigenic role of NO in cancer through the identification of NO-driven molecular mechanisms that promote disease progression. Moreover, we propose to utilize this mechanistic information for the development of novel drugs that target specific biomarkers, or combinations of biomarkers, for improved therapeutic response. One inflammatory signaling loop that we are currently examining involves elevated NOS2/COX2 (cyclooxygenase-2) expression through the engagement of toll-like (TLR) receptors. Bacterial lipopolysaccharide (LPS) stimulation of TLR4 promotes tumor metastasis through NF- $\kappa$ B-dependent upregulation of NOS2, matrix metalloproteinase-2 (MMP-2), and  $\beta$ 1 integrin. In addition, LPS activation of tumor cell TLR4 increases NO production, as well as IL-6 and IL-12, and facilitates tumor evasion from immune surveillance. In collaboration with Dr. Stefan Ambs at the Bethesda campus, we have found that elevated NOS2 and/or COX2 predict poor survival in estrogen receptor negative (ER-) breast cancer patients. Using cell culture models, we have shown that NO induces COX2, and PGE2 induces NOS2 leading to a feed-forward signaling mechanism. Furthermore, elevated tumor NOS2 is associated with increased S100A8 expression, which is a TLR4 agonist. Steady state NO flux > 200 nM upregulates S100A8 expression in ER- breast cancer cells suggesting that this NO flux may promote this feed-forward loop. Importantly, NOS2 pharmacological inhibition reduces IL-6 and S100A8 biomarker expression, tumor growth and dramatically abates lung and brain metastases in animals bearing ER- breast tumors. Together, these results demonstrated mechanistic biomarkers for unresolved inflammatory signaling loops in the tumor microenvironment that may be pharmacologically targeted to improve patient therapeutic response and survival. We propose to test the anti-tumor effects of NOS3 inhibitors and novel NSAIDs designed and synthesized by our collaborators for their abilities to suppress the above biomarkers and reduce tumor growth and metastases in mice. A motion for a conditional approval, pending clarifications and an observation, was made by Sharon Altman and Bradley St Croix. (For: 11; Against: 0; Abstain: 0)

- ❖ Drs. Pavlakis and Felber – 15-13 (07-01): Use of lentiviral/retroviral vectors for gene transfer into mammalian cells. The objective is to use lentiviral/retroviral vectors as vehicle for gene transfer into mammalian cell lines. We use this system to insert a gene of interest into the packaging vector, generate pseudotyped virions and generate stable modified cell lines. The advantage of using these systems is that only a few copies of a gene of interest are integrated. Lentiviral/retroviral vector systems consists of 3 independent plasmids expressing (a) the gene of interest such as cytokines, cytokine receptors, HIV/SIV genes; (b) the packaging signal and the marker gene like luciferase or Green Fluorescent protein GFP and/or a selection marker like neomycin; (c) the gene for one single round of replication such as env (VSV-G to enter any cells). For this reason, the pseudotyped virions are only competent for a single round of infection. The separation of the packaging signal, LTRs and gag/pol and env genes into separate plasmids eliminates the chance of recombination. The plasmids are obtained either from other investigators or are generated by us. A combination of the respective plasmids is transiently transfected into

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human 293 cells (this work is performed in the BSL-2\* facility) and the supernatant is directly used to infect the cell line of interest such as HEK293 and primary murine cells. We generate stable cell lines (i.e. selecting for neo resistant cells), generating i.e. cell lines expressing the co-receptors CCR5, CXCR4, cytokine, cytokine receptors, or any gene of interest. ***A motion to defer this registration to schedule a meeting with the PI was made by Serguei Kozlov and seconded by Theresa Bell at the November meeting. A meeting was held with the lab to discuss the clarifications needed for approval. The meeting clarified all changes to be made in this registration.*** (For: 11; Against: 0; Abstain: 0)

**OUTSTANDING ITEMS**

- ❖ Ji Ming Wang – 14-46: The role of mouse mFPR2 in the pathogenesis of Helicobacter Pylori. H.pylori infects human stomach to cause inflammation and sometime H.pylori produces peptides that activate a G-protein coupled receptor FPR2 in human and mFPR2 (in mouse, also termed Fpr2) to induce migration of neutrophils and monocytes, therefore may establish a basis for inflammation. The purpose of this proposal is to use mice deficient in Fpr2 to examine their susceptibility to H.Pylori-induced stomach inflammation and potential cancer. A motion to approve with the clarification that a mock observation is to be performed before work begins. ***February 2, 2016, email from PI that he is still awaiting the approval of a staff scientist who will be conducting this study. PI has put this observation on hold due to new staffing and the PI to return to the country. A mock observation to be performed before work can commence.***

**AMENDMENTS**

Twenty nine amendments were processed and approved between January and February IBC meetings.

**OTHER BUSINESS**

**ADJOURNMENT**

The meeting adjourned at 12:45 pm.

**Next meetings:            February 16, 2016            March 15, 2016**