

NATIONAL CANCER INSTITUTE AT FREDERICK (NCI@F)
INSTITUTIONAL BIOSAFETY COMMITTEE
MINUTES
MARCH 17, 2015

CALL TO ORDER / ANNOUNCEMENTS

The NCI at Frederick Institutional Biosafety Committee was convened at 12:05 pm in Building 549 Executive Board Room with the following members in attendance:

Voting (Quorum = 8)

Michael Baseler (*regrets*)
 Theresa Bell
 Rev. David Betzner
 Stephen Creekmore
 Bruce Crise
 Eric Freed
 Melinda Hollingshead
 Stephen Hughes

Sarah Hooper
 Serguei Kozlov (*regrets*)
 Dan McVicar
 Raja Sriperumbudur (*regrets*)
 Lucien Winegar (*regrets*)
 Sharon Altmann
 Patti Labbe

Non-Voting

Walter Hubert
 Karen Barber
 Matthew Nawn

APPROVAL OF MINUTES FROM THE FEBRUARY 24TH MEETING

The minutes from the February 24, 2015 meeting were approved. A motion and second were made. (For: 10; Against: 0; Abstain: 0)

ACCIDENT REVIEWS : Nothing to report

REVIEW OF PROTOCOLS

NEW REGISTRATIONS

- ❖ Esta Sterneck – 15-07: Silencing of CEBPD in patient-derived glioblastoma tumor cell lines. Recent studies from our laboratory have illustrated that CEBPD promotes tumor metastasis in an MMTVNeu tumor mouse model. CEBPD exerts its prometastatic activity in part through hypoxia adaptation and proinflammatory signaling. Our current projects aimed at understanding role of CEBPD in cancer stem-like cells (CSCs) which promote metastasis. We found that CEBPD promotes stemness in breast tumor cells, primary mouse tumor cells and xenografts through regulation of stemness factors. Interestingly, CEBPD expression is highly elevated and functions as a critical driver for glioblastoma tumor progression. To address the relevant function of CEBPD in maintaining cancer stem cells also in glioblastoma the goal is to knockdown endogenous CEBPD in glioblastoma patient-derived tumor cell lines and see the effect of CEBPD silencing on cell proliferation, stemness factors expression. The following manipulations may be conducted: lysis of

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cells for preparation of mRNA and protein (RNA analysis, Western blot analysis). A motion to approve with minor edits to the registration was made by Theresa Bell and seconded by Sharon Altmann. (For: 10; Against: 0; Abstain: 0)

RENEWAL REGISTRATIONS

- ❖ Amy Hutchinson – 14-47: DNA Extraction & Staging Lab (DESL)- The DNA Extraction and Staging Laboratory is responsible for extraction of nucleic acids (DNA and RNA) from a variety of human-derived source materials including but not limited to whole blood, various blood products, saliva, and tissue (both frozen and fixed). DNA is then QC's and prepared using an automated staging pipeline for use in downstream applications such as sequencing and genotyping. ***A motion was made to defer to the April meeting with further clarifications.*** (For: 10; Against: 0; Abstain: 0)

- ❖ Glenn Merlino – 15-04 (11-26): Role of receptor tyrosine kinases in melanoma and other cancers: Use of genetically engineered mice. Our program revolves around the generation of mouse models of cutaneous malignant melanoma and to a lesser extent non-small cell lung cancer (NSCLC) and rhabdomyosarcoma, which we use to study the role of receptor tyrosine kinase and other signaling pathways in cancer initiation and progression. Genetically engineered mice (GEM) are generated using rDNA. Also, isolated cultured cells are genetically manipulated by insertion of naked DNA or retroviral/lentiviral agents, and then transplanted into mice to study tumor formation and metastasis. The vast majority of the lentiviruses will be ordered from the CRTP, Leidos, and occasionally we will run lentiviral packaging in building 37, Room 4032C1 (BSL2 cell culture room specifically for virus work). Production of retrovirus and viral transduction of the cells will be performed in the BSL2 lab in Bethesda. All animal studies will be performed in Frederick. A motion to approve pending clarification to a sub-committee was made by Bruce Crise and seconded by Theresa Bell. (For: 10; Against: 0; Abstain: 0)

- ❖ Denise Whitby – 15-05 (09-09): Epidemiology genetic, and immunological factors of Kaposi Sarcoma Herpes virus transmission. Kaposi's Sarcoma Herpesvirus will be studied, examining the epidemiological risk factors associated with virus transmission, the molecular biology of virus / host interaction, and cellular and immunological mechanisms of viral infection. Some of this work will involve the use of purified virus to infect cell cultures using established cell lines or primary cells isolated from humans. Plasmids and vectors expressing KSHV viral gene products will also be used to transfect specific cell lines and purification of viral proteins. Additional details about the specimen tests and herpesvirus research is described below:
 1. Specimen Testing: Epidemiological / clinical samples are tested for oncogenic viruses (KSHV, EBV, HCV, and HBV) using various serological and molecular biology techniques. The serological techniques employed are ELISA, western blot, RIBA, Luminex, ELISPOT, and IFA. The molecular techniques include nucleic acid extraction, real-time quantitative PCR, sequencing, and gene chip analysis.
 2. Herpesvirus Research: Single or double infected (KSHV and/or EBV) cell lines are propagated and induced to produce virus. The virus-containing cell supernatant is harvested and virus is concentrated. Virus concentrate is used to infect naive cells or for biochemical analyses. A

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motion to approve, pending clarifications to the lead reviewers, was made by Eric Freed and seconded by Bruce Crise. (For: 10; Against: 0; Abstain: 0)

- ❖ Cheryl Winkler – 15-06 (10-17): Genetics of Complex Diseases. We use RNA, DNA, and human samples to identify genes and variation associated with human diseases. Deferred to April meeting. Awaiting further documentation from the PI. (For: 10; 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 a 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 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. Deferred to April meeting. Awaiting further documentation from the PI. (For: 10; Against: 0; Abstain: 0)

- ❖ Jeffrey Green – 15-15 (06-56): Genetically engineered and xenograft models of mammary cancer. The objectives of this project are to understand mechanisms of mammary cancer development and metastases through the use of genetically-engineered model systems and xenograft models. Several lines of transgenic and knock-out mice are used for this purpose. Additionally, mouse and human cell lines will be injected into syngeneic or nude mice to determine tumor growth potential or metastatic potential of the cell lines. The cell lines may be transfected or transduced to express luciferase or GFP in order to follow growth of tumor cells. A motion to approve pending clarifications to a sub-committee was made by Dan McVicar and seconded by Theresa Bell. (For: 10; Against: 0; Abstain: 0)

- ❖ Ven Natarajan – 11-33-A2: adding SEB to this registration. This registration was brought to full committee due to the select agent. LMCB is interested in studying the role of protein LZTFL1 in immune synapse formation. When a T cell comes in contact with an antigen-presenting cell, synapse is formed at the contact site and materials and signals are exchanged between the two cells through the synapse. Staphylococcal enterotoxin B (SEB) has been shown to be a super-antigen, strongly activating oligo clonal populations of T lymphocytes. Hence this toxin is an ideal candidate to prepare antigen-presenting cells that would activate a large population of T cells. Raji

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B cells will be treated with SEB and mixed with equal number of Jurkat cells. These cells will be treated with 4% formaldehyde and stained with primary and secondary antibodies and observed under microscope for immune synapse formation. A motion to approve was made by Dan McVicar and seconded by Theresa Bell. (For: 10; Against: 0; Abstain: 0)

OUTSTANDING ITEMS

- ❖ Peter Gorelick – 14-26 (08-27): Serological diagnostic testing of non-human primates for the presence of potentially adventitious viruses - Diagnostic serological testing for routine health monitoring of NHPs. (Bell) **Deferred to full committee in August. Awaiting additional documentation.**
- ❖ Stephen Lockett – 14-22 (08-46): Ras project 3 and CCR support. Discovery methods to directly target oncogenic Ras protein, and live and fixed cell fluorescence labeling in support of CCR research. (Zudaire/Hughes/Altmann) Deferred to full committee in August. Awaiting additional documentation. **ON HOLD. WAITING ON CHANGES IN THE DEPARTMENT BEFORE SUBMITTING. As of October 14, 2014, no updates have been made.**
- ❖ Dimiter Dimitrov 13-38 (04-04, 08-20): Developing anti-viral vaccines and human antibodies against infectious diseases and cancer antigens by using recombinant membrane proteins of HIV, Nipah, Hendra, Dengue viruses and cancer antigens. Committee requested additional clarifications and a Vaccinia-specific SOP as well as a lab visit. Post-meeting, Theresa Bell learned that the lab was relocating and suggested that the space that will be used for the Vaccinia work should not be evaluated until the move has been completed. No Vaccinia work is being performed at this time. **Approved. Need to visit lab space once moved for Vaccinia work.** For: 8; Against: 0; Abstain: 0

AMENDMENTS

Twenty amendments were processed and approved between February and March IBC meetings.

OTHER BUSINESS

ADJOURNMENT

The meeting adjourned at 1:15 pm.

Next meetings:

April 21, 2015

May 19, 2015