

**NATIONAL CANCER INSTITUTE AT FREDERICK (NCI@F)**  
**INSTITUTIONAL BIOSAFETY COMMITTEE**  
**MINUTES**  
**DECEMBER 15, 2015**

**CALL TO ORDER / ANNOUNCEMENTS**

The NCI at Frederick Institutional Biosafety Committee was convened at 12:00 pm in Building 426 Conference Room with the following members in attendance:

Voting (Quorum = 8)

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

- Sarah Hooper
- Serguei Kozlov
- Dan McVicar
- Bradley St. Croix
- Lucien Winegar (*regrets*)
- Sharon Altmann
- Robin Sun
- Jatinder Gulani

Non-Voting

- Walter Hubert
- Karen Barber

Visitors

Ted Witte  
Antonio Valentin

**APPROVAL OF MINUTES FROM THE SEPTEMBER 15 MEETING**

The minutes from the November 17, 2015 meeting were approved. A motion to approve and a second were made. (For: 11; Against: 0; Abstain: 0 )

**ACCIDENT REVIEWS :**

None

**REVIEW OF PROTOCOLS**

***NEW REGISTRATIONS***

- None presented.

***RENEWAL REGISTRATIONS***

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- ❖ Ying Zhang – 15-54 (12-81): Tumor models to study TGF-beta/Smad signaling. The goal of our project is to understand the role of TGF-beta, particularly, its downstream regulators, TGF-beta receptors, Smads, Smurfs in tumorigenesis and cancer metastasis. We will use cell lines that overexpress either cDNA or shRNA. These cell lines are/will be established using a retroviral delivery system. It is based on Moloney Murine Leukemia Virus (MoMuLV) or Murine Stem Cell Virus (MSCV) and allows for delivery of genes to most dividing mammalian cell types. These cell lines generated in our lab in Bethesda will be injected into nude mice in our Frederick animal facility. Results from these studies will help us to better understand underlying mechanism of tumorigenesis and tumor progression, shed light for devising novel therapeutic strategies. A motion to approve with minor clarifications was made by Dan McVicar and seconded by David Betzner. (For: 11; Against: 0; Abstain: 0)
- ❖ Robin Dewar – 15-55 (11-60): Virus isolation and Serology Laboratory: Work with Clinical Specimens. One of the main functions of the Virus Isolation and Serology Laboratory (VISL) is to test clinical specimens from patients with HIV disease or other emerging/re-emerging infectious diseases during their treatment with a variety of antiviral and immunomodulatory agents. To do this, the laboratory receives whole blood, leukapheresed packs, serum, blood plasma, purified PBMC, CNS fluid and biopsies of the gut and lymph nodes for testing. Testing procedures include: Serologic assays to determine seroreactivity to viral proteins, Viral Load assays that quantify virus concentrations in a variety of clinical samples, and Genotyping assays that identify viral subtypes and drug resistance mutations. These procedures require pipetting and centrifugation of clinical samples. A motion to approve with clarifications and a mock observation was made by Steve Creekmore and seconded by Robin Sun. (For: 11; Against: 0; Abstain: 0)
- ❖ Paul Roche – 15-56 (12-48): Breeding and Maintenance of Mice. **Notification only.** Breeding protocol. (Approved by Hollingshead)
- ❖ Vanja Lazarevic – 15-57 (12-41): Breeding, maintenance and genotyping of mouse colonies. Breeding protocol. (Approved by Kozlov)
- ❖ Andre Nussenzweig – 15-58 (11-61): Role of DNA repair in tumorigenesis. Breeding protocol (Approved by Hollingshead)
- ❖ Bradley St Croix – 15-59 (12-29): Generation of DNA vectors for functional analysis of various endothelial markers. Tumor Endothelial Markers (TEMs) are genes that are expressed in the endothelial cells that line proliferating tumor blood vessels. Most of the experiments we plan to do involve basic cloning of endothelial genes in various vectors designed for the propagation and expression of TEMs in either E.coli or mammalian cells. The studies we are proposing can be grouped into six projects:
  - Project 1: The goal of this project is to generate various recombinant DNA vectors, all for in vitro use that will aid in the identification of binding partners for various endothelial cell surface receptors and enable us to better understand the expression patterns and function of the proteins being studied. Sources of cDNA inserts will include premade cDNA libraries

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- (Clontech), commercially available cDNAs or ESTs (IMAGE consortium) and collaborators. To monitor RNA expression by non-radioactive in situ hybridization, cDNAs will be used to construct riboprobes. Recombinant proteins will be made by inserting genes into commercially available bacterial or mammalian expression vectors.
- Project 2: The goal of this work is to determine if tumor endothelial markers such as TEM8 or CD276 are potentially useful stromal targets for the development of antibody drug conjugates (ADCs). For this study, anti-TEM ADCs conjugated to the small molecule drugs MMAE or PBD will be tested for anti-tumor activity in vivo. To test the specificity of the antibodies for their targets, some of the experiments will be performed in gene knockout models in our laboratory, such as TEM8<sup>-/-</sup> or CD276<sup>-/-</sup> mice.
  - Project 3: The goal of this work is to use lentiviral particles to obtain high level gene expression in cell lines in culture. For example, beta-catenin responsive luciferase reporters will be transduced into brain endothelial cells or 293 cells to generate reporter cells that can be used to monitor beta-catenin signaling. In other experiments shRNA will be delivered into cells using lentiviral particles to knock down gene expression. The lentiviral vectors we wish to use would be obtained from SABiosciences or Sigma. No in vivo research with lentiviral vector will be performed.
  - Project 4. We plan to use antibodies which we have generated against cell surface TEMs to develop ELISA based assays to detect blood markers of angiogenesis. For this assay, serum or plasma derived from cancer patients or normal controls will be used in an ELISA. An anti-TEM antibody will be coated on a 96-well plate and used to capture TEM proteins from the plasma, serum or blood, and an independent antibody will be used to measure the amount of bound TEM protein.
  - Project 5. We are working with endothelial cells isolated from various conditional knockout strains, for example, mice that contain the TEM8 or CD276 genes flanked by lox-p sites. To explore the function of each of these genes in vitro, we would like to delete the genes (separately) from these cells in tissue culture by exposing the appropriate cells to adenoviral-Cre. We would like to use adenoviral vectors to deliver CRE recombinase because of their high infection efficiency in endothelial cells. The adenoviral vectors expressing CRE will be obtained from the Viral Technology Laboratory (VTL) at NCI-Frederick or from Vector Biolabs. No in vivo research with adenoviral vectors will be performed.
  - Project 6. In order to understand the expression patterns of various molecules we study in the context of cancer, we plan to perform immunofluorescence staining on frozen tissues derived from various normal human tissues or tumors. (Hughes/Freed/Gulani)

A motion to approve with clarifications was made by Dan McVicar and seconded by Serguei Kozlov. (For: 10; Against: 0; Abstain: 1).

- ❖ 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

