

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

**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 (regrets)     |
| <input type="checkbox"/> Stephen Creekmore (regrets)    |  |
| <input checked="" type="checkbox"/> Bruce Crise         | <input checked="" type="checkbox"/> Lucien Winegar |
| <input type="checkbox"/> Eric Freed                     | <input type="checkbox"/> Sharon Altmann (regrets)  |
| <input type="checkbox"/> Melinda Hollingshead (regrets) | <input checked="" type="checkbox"/> Robin Sun      |
| <input type="checkbox"/> Stephen Hughes                 |  |

Non-Voting

- Walter Hubert
- Karen Barber

Visitors

Sam Denny  
Ted Witte  
Gillian Braden-Weiss

**APPROVAL OF MINUTES FROM THE AUGUST 18 MEETING (NO JULY IBC MEETING)**

The minutes from the August 18, 2015 meeting were approved. A motion to approve and a second were made. (For: 8; Against: 0; Abstain: 0 )

**ACCIDENT REVIEWS :**

Animal Bite with transgenic mouse  
NHP – Bethesda – B virus protocol for this individual  
Sharps safety assessment – redstone - completed

**REVIEW OF PROTOCOLS**

***NEW REGISTRATIONS***

- ❖ Vinay Vyas – 15-40: Production of EBV gp350-Ferritin nanoparticle (gp350-FN) using HEK293s. The BDP has a proven track record of partnering with clients to develop difficult biologic products for preclinical research and clinical trials. The BDP/FNLCR is a cGMP-compliant facility that offers

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distinct capabilities in product feasibility to development, manufacturing, testing, QA oversight, and regulatory documentation. The BDP received this request from NIAID to develop and produce EBV Gp350-Ferritin (Epstein-Barr virus cell surface associated glycol-protein 350), virus-like particle (VLP), for preclinical and human clinical studies. Work scope will include developing a manufacturing process and related product release assays to produce EBV Gp350-Ferritin VLP. Product would be manufactured to support reference standards, preclinical studies, stability studies, and clinical studies. Analytical methods like HPLC-SEC would be established for product quality and release. Research on the production of gp350-Ferritin nano-particle has been previously approved under NIH IBC RD 13 X-01. A motion to approve was made by Bruce Crise and seconded by Theresa Bell (For: 8; Against: 0; Abstain: 0)

- ❖ Simone Difilippantonio – 15-41: Rederivation and breeding of germ free mice. Over the last few years, research addressing the role of microbiota in inflammation, pathogenesis and antitumor response has evolved. This research must be conducted on germ-free animals that are inoculated with a defined microflora and housed in Gnotobiotic Animal facilities. Maintenance of germ-free isolators including environmental monitoring, management of germ-free breeding colonies and the performance of experimental procedures in isolators requires specialized knowledge, training and experience. LASP has recently established a Gnotobiotic Facility and is providing technical services in support of experimental studies on germ-free animals. To assist investigators in obtaining germ-free animals for their studies, LASP is rederiving desired mouse strains into the germ-free status. In order to produce cohorts of experimental colonies, the rederived animals will be bred in the Gnotobiotic facility. Experimental studies conducted on these animals will be covered under the animal study proposals of the requesting investigator. A motion to approve was made by Bruce Crise and seconded by Sarah Hooper. (For: 8; Against: 0; Abstain: 0)
- ❖ Tetsuro Kobayashi – 15-42: Analysis of exzematous mouse models. Atopic dermatitis (AD) is a common skin disorder in clinical practice. Although AD is not an infectious disease, it is frequently associated with increased colonization of *Staphylococcus aureus*, which is thought to contribute to the disease pathogenesis. We have recently developed a mouse model (Adam17flox/flox/Sox9Cre mice; Adam17/Sox9 mice) that spontaneously developed eczematous dermatitis, with intense pruritus, increased barrier dysfunction and increased serum IgE. In this study, we aim to identify the molecular mechanism by which dysbiosis emerges and dysbiosis leads to inflammation. To reveal the direct association between dysbiosis and eczema, we will generate germ free Adam17/Sox9 mice and *S.aureus* inoculated gnotobiotic Adam17/Sox9 mice. We will examine skin abnormalities that cause dysbiosis in germ free mice and leukocytes that drive exzematous dermatitis in gnotobiotic Adam17/Sox 9 mice. A motion to approve pending clarifications was made by Bruce Crise and seconded by Serguei Kozlov. (For: 8; Against: 0; Abstain: 0)
- ❖ Kathryn Muegge – 15-43: Induction of transcription factors in embryonal fibroblast cell lines. The objective of this proposal is to study epigenetic states that determine transcription factor binding, gene expression and ultimately the phenotype of a cell. Using a direct reprogramming approach we can induce neuronal, hepatic or macrophage cell type specific lineage markers in fibroblasts. The seven expression vectors that encode distinct murine transcription factors, will be delivered to

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**SEPTEMBER 15, 2015**

murine embryonic fibroblast (MEF) cell cultures. Stable expressing cell lines will be selected. A motion to approve pending clarifications was made by Serguei Kozlov and seconded by David Betzner. (For: 8; Against: 0; Abstain: 0)

***RENEWAL REGISTRATIONS***

- ❖ 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 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 to the October meeting for further clarifications was made by Serguei Kozlov and seconded by Theresa Bell.*** (For: 8; Against: 0; Abstain: 0)
  
- ❖ Dan McVicar – 15-39(11-64): Retroviral Manipulation of Metabolism and Inflammation. Initial response to pathogens is regulated by cell of the innate immune system. This system includes macrophages, dendritic cells, and Natural Killer (NK) cells. Our laboratory studies the regulation of immune response in these cells by a variety of immune regulatory receptors and by enzymes in metabolic pathways. The main emphasis of this work is the study of the signal transduction apparatus of innate immune cells and how it translates into metabolic regulation of immunity. Toward this end, this proposal involves the use of Lenti- and Retroviral-based vectors for the expression of genes and/or specific gene silencing using shRNA (small sequences of RNA that lead to destruction of endogenous RNA and down regulation of gene expression). The experiments based on three approaches:
  1. The first involves the expression of chimeric and other forms of innate immune receptors and/or metabolic enzymes in primary innate immune cells and on cell lines derived from mice some of whom may carry mutations in signal transduction proteins or metabolic enzymes. In these experiments, cells from mice are purified in vitro before culture and transduction with recombinant viral vectors. (Vectors here may be lentiviral or derived from murine retrovirus.) Biochemical, immunological, and metabolic function are then evaluated in vitro. We do not

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**MINUTES**  
**SEPTEMBER 15, 2015**

anticipate re-introduction of transduced cells into mice at this time. Oncogenes are not expressed, nor are protooncogenes.

2. The second approach involves targeted disruption of receptors, signaling proteins, or metabolic enzymes using viral vectors carrying shRNA constructs. In most cases primary cells isolated from mouse tissue or human blood will be infected in vitro, then cultured and assessed in vitro for immune, biochemical and/or metabolic function. In other cases, retroviral particles are injected I.P. to directly infect resident macrophages of mice. Several days after transduction cells are harvested by lavage and evaluated in vitro for immune, biochemical and/or metabolic function. All in vivo transductions will be using Lenti-Viral vectors so as to minimize the possibility of complementation of endogenous murine retrovirus. Tumor suppressor genes are never targeted.
3. The third approach involves the expression of Cre via Adenoviral vectors produced within the PEL. Cells isolated from mice with flexed alleles of signaling proteins, receptors, or metabolic enzymes are infected in vitro with Adeno-Cre. The cells are cultured after infection then assessed for immunologic, biochemical, or metabolic function. In some cases, adenoviral particles are injected IP to mediate deletion of genes in the resident macrophage populations. After injection, cells are removed from the animals and assessed as above.

A motion to approve was made by Mike Baseler and seconded by Bruce Crise. (For: 8; Against: 0; Abstain: 0)

- ❖ Howard Young – 15-33 (12-02): Analysis of leukocyte gene expression. The laboratory analyzes leukocyte gene expression in response to many different types of stimuli. Two sets of approaches are used in the lab with two distinct sets of safety concerns: 1) This work involves the generation of cell lines from mice using a retrovirus carrying the Myc and Raf oncogenes; 2) this work involves the in vitro manipulation of murine NK cells, macrophages or T cells. A motion to conditionally approve pending clarifications was made by Theresa Bell and seconded by Serguei Kozlov. (For: 8; Against: 0; Abstain: 0)
- ❖ Ligia Pinto – 15-35 (12-34): Human Papillomavirus antibody assays using serum, saliva, cervical secretions, and other body fluids. The object of this proposal is to evaluate the activity of antibodies in serum, cervical secretions and other body fluids to neutralize HPV in vitro and to evaluate anti-HPV titers and avidity in biological samples (serum, saliva, cervical secretions and other body fluids). A motion to defer this to the October meeting was made by Theresa Bell and seconded by Serguei Kozlov. (For: 8; Against: 0; Abstain: 0)
- ❖ Ligia Pinto – 15-36 (12-33): Cellular immunity studies using mononuclear cells originated from blood or bone marrow. The purpose of this proposal is to describe the cellular immunity assays used by the HPV laboratory to evaluate the human cellular immune response to available HPV vaccines. A motion to defer this to the October meeting was made by Theresa Bell and seconded by Serguei Kozlov. (For: 8; Against: 0; Abstain: 0)

**OUTSTANDING ITEMS**

**NATIONAL CANCER INSTITUTE AT FREDERICK (NCI@F)**  
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**MINUTES**  
**SEPTEMBER 15, 2015**

- ❖ 15-21 – Joost Oppenheim: Chemoattractant and chemoattractant receptor structure/function studies. The goal of our research is to better understand the role of chemoattractants in the initiation and prevention of diseases, specifically cancer and autoimmunity. Construction and expression on prokaryote and eukaryote expression vectors will be undertaken to express human and mouse chemoattractant and/or chemoattractant receptors in common cell lines. These vectors will be expressed in prokaryote or eukaryote cell lines for functional studies in vitro. pcDNA based constructs that demonstrate expression and function may be injected into mice by electroporation or direct injection for evaluation as preventatives. In order to evaluate signaling between cellular receptors primary mouse fibroblasts from commercially produced Tg or KO mouse strains will be immortalized using standard procedures. Currently we utilize commercially available or previously produced Tg or KO mouse strains and do not plan to produce our own. ***Deferred to the October meeting for mock observation.***
  
- ❖ 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. ***Deferred to the October meeting by PI for further clarifications.***
  
- ❖ 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. **PI has put this observation on hold due to new staffing.**

**AMENDMENTS**

Twenty one amendments were processed and approved between August and September IBC meetings.

**OTHER BUSINESS**

**ADJOURNMENT**

The meeting adjourned at 1:30 pm.

***Next meetings:                      October 20, 2015                      November 17, 2015***