

NATIONAL CANCER INSTITUTE AT FREDERICK (NCI@F)
INSTITUTIONAL BIOSAFETY COMMITTEE
MINUTES
AUGUST 18, 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 type="checkbox"/> Michael Baseler (<i>regrets</i>) | <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 checked="" type="checkbox"/> Dan McVicar |
| <input checked="" type="checkbox"/> Stephen Creekmore | <input checked="" type="checkbox"/> Raja Sriperumbudur |
| <input checked="" type="checkbox"/> Bruce Crise | <input checked="" type="checkbox"/> Lucien Winegar |
| <input checked="" type="checkbox"/> Eric Freed | <input type="checkbox"/> Sharon Altmann (<i>regrets</i>) |
| <input checked="" type="checkbox"/> Melinda Hollingshead | <input checked="" type="checkbox"/> Robin Sun |
| <input checked="" type="checkbox"/> Stephen Hughes | |

Non-Voting

- Walter Hubert
- Karen Barber
- Ted Witte
- Gillian Braden-Weiss

Visitors

Sam Denny
Simone Difilippantonio

APPROVAL OF MINUTES FROM THE JUNE 16 MEETING (NO JULY IBC MEETING)

The minutes from the June 16, 2015 meeting were approved. A motion to approve and a second were made. (For: 13; Against: 0; Abstain: 0)

ACCIDENT REVIEWS :

Laceration – 7/1/15 at 10:15 a.m. – Cut finger on a clean cryostat blade – low profile microtome blade

Mouse bite – 7/8 at 2:09 pm – transgenic mouse, 01/34 IFN-Gamma Cre 1821, on ASP 14-018 was being bred at the time of the incident.

Laceration – 7/15/15 at 2:13 p.m. – NCI student intern lacerated left thumb while operating a microtome, shaving a wax formalin-fixed paraffin-embedded (FFPE) sample.

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REVIEW OF PROTOCOLS

NEW REGISTRATIONS

- ❖ Joseph Barchi – 15-26: Antimetastatic activity of tumor-associated carbohydrate antigen-coated gold nanoparticles in a highly metastatic breast cancer mouse model. The object of this project is to determine the biodistribution in mice, effectiveness and antimetastatic properties of several gold nanoparticle (AuNP) formulations where the surface is coated with a tumor-associated carbohydrate molecule. These molecules are presented on tumor cells differently than normal cells, and are targets of the immune system, hence their broad use in anticancer vaccine preparations. However, the specific molecule we study, the Thomsen Friedenreich (TF) antigen has a special dual purpose, where this structure is directly related to metastatic spread of various tumors via it's interaction with another molecule that specifically binds to it called Galectin-3 (Gal-3). The purpose of these studies is to demonstrate that the certain presentations are more selective than others. Initial experiments study the antimetastatic effect and both tumor –specific and global biodistribution of our AuNPs in the 4T1-luciferase mouse model. In addition, toxicity of each treatment will be assessed after each study via blood panels and histological analysis of the organs in the treated mice. A motion to conditionally approve pending clarifications to a subcommittee was made by Steve Hughes and seconded by Steve Creekmore (For: 13; Against: 0; Abstain: 0)

- ❖ Vanja Lazarevic – 15-30: Transcriptional regulation of CNS autoimmune responses. Our laboratory studies the events that lead to the breakdown of immunological tolerance and development of autoimmune diseases. We use genetically modified mice (transgenic and targeted gene knockout mice) to identify the role of specific genes in these processes. A motion to approve was made by Steve Creekmore and seconded by Dan McVicar. (For: 13; 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 vectors 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

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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 September meeting for further clarifications was made by Serguei Kozlov and seconded by Steve Hughes.*** (For: 11; Against: 0; Abstain: 0)

- ❖ Ronald Gress – 15-27 (12-51): **Breeding only**, holding of and procedures (thymectomy) for various mouse strains. We plan to continue to breed and hold mice as described in ASP 15-435 for export to approved facilities for analysis of immunological function and T cell homeostasis, development and repertoire. None of the mice at NCI-Frederick will be treated with infectious agents. ***Approved by lead reviewer.***
- ❖ Bruce Shapiro – 15-29 (11-08): Experimental determination of RNA structure and function based on computational predictions. The major focus of the RNA Structure and Design Section is the research and development of computational methodologies for understanding RNA structure and function, and utilizing these methods to design and deliver RNA-based nanoparticles. A motion to conditionally approve pending clarifications was made by Eric Freed and seconded by Bruce Crise. (For: 13; Against: 0; Abstain: 0)
- ❖ Esta Sterneck – 15-31: Analysis of the Roles of CEBPD in Inflammatory Breast Cancer. The CEBPD transcription factor promotes tumor metastasis in mouse models of mammary tumorigenesis in part through hypoxia adaptation and pro-inflammatory signaling. A motion to conditionally approve pending clarifications was made by Melinda Hollingshead and seconded by Theresa Bell. (For: 13; Against: 0; Abstain: 0)
- ❖ Thomas Sayers – 15-32(11-35): Modification of tumor antigenicity and cell death in vitro and in vivo. Project 1: the objective of these studies is to assess how the immune system promotes tumor destruction in vitro and in vivo at the molecular level. In order to examine this mouse tumor cells will be modified by gene transfer to express specific antigens such as the viral antigen hemagglutinin (HA). Project 2: recent work from our laboratory indicates that mouse and human cancer cell lines contain a small subpopulation of cells that express the embryonic transcription factors Nanog, Oct4 or Sox2. A motion to defer to the September meeting was made by Serguei Kozlov and seconded by Steve Hughes. (For: 12; Against: 0; Abstain: 1)
- ❖ 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. **UPDATES TO BE MADE BY PI. DEFERRED TO SEPTEMBER MEETING.**
- ❖ Ligia Pinto – 15-34 (12-38): Holding protocol for recombinant Adenovir5us, Vaccinia Virus and EBV in the HPV laboratory. The purpose of this proposal is to document storage of recombinant Vaccina, recombinant Adenovirus, Epstein Barr Virus (EBV) supernatants and EBV transformed B

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cells from healthy HPV vaccine recipients. A motion to approve was made by Dan McVicar and seconded by David Betzner. (For: 13; 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 September meeting was made by Dan McVicar and seconded by Theresa Bell. (For: 13; 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 September meeting was made by Dan McVicar and seconded by Theresa Bell. (For: 13; Against: 0; Abstain: 0)
- ❖ Ligia Pinto – 15-37 (12-35): Effects of soluble factors produced by PBMC on HPV expression. ***PI inactivated this registration and moved the W12 cell line to their holding protocol 14-42.*** (Hughes/Kozlov)
- ❖ Barbara Felber – 15-38 (06-86, 06-89): Studies using infectious SIV/HIV isolates and SIV/HIV molecular clones. We study the effects of cytokine treatment in the viral (HIV and SIV) replication using primary lymphocytes from humans (healthy blood donors) and macaques. We perform infections in vitro and compare the changes in the cell phenotype and the propagation pattern of the virus in samples that are treated with several cytokines. ***Deferred to the September meeting for further clarifications from the PI.***

OUTSTANDING ITEMS

- ❖ 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 September meeting by PI for further clarifications.***
- ❖ 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

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research. (Zudaire/Hughes/Altmann) Deferred to full committee in August. Awaiting additional documentation. ***Deferred to the September 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 until June 2015 due to new staffing.**

AMENDMENTS

Forty-five amendments were processed and approved between June and August IBC meetings.

OTHER BUSINESS

ADJOURNMENT

The meeting adjourned at 2:10 pm.

Next meetings: September 15, 2015 October 20, 2015