NCI-FREDERICK
INSTITUTIONAL BIOSAFETY COMMITTEE

Minutes
September 15, 2009
NCI-Frederick

INTRODUCTION

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

Dr. Dan McVicar, Chair; Ms. Theresa Bell, IBC Secretary and Biosafety Officer; Dr. David Garfinkel; Dr. Serguei Kozlov; Ms. Dianna Conrad; Mr. Lucien Winegar; Dr. Stephen Hughes; Dr. Randall Morin; Dr. Michael Baseler; Dr. Eric Freed; Dr. David Derse; Dr. Stephen Creekmore

Members not in attendance: Dr. Bruce Crise; Reverend David Betzner; Dr. Melinda Hollingshead; Mr. Scott Jendrek; Dr. Henry Hearn; Ms. Alberta Peugeot

Others in attendance: Dr. Scott Keimig

MINUTES

Dr. McVicar asked for edits to the posted meeting minutes. Hearing none, Dr. McVicar made a motion to approve meeting minutes posted on sharepoint for May, June and July 2009. Ms. Bell seconded and all were in favor.

ACCIDENT REVIEWS

Mary Carol Fleming (attending on behalf of OHS) presented the accident cases for the previous month.

The first discussion involved an accident with a scalpel. An employee was in the process of changing a scalpel blade and cut their hand. The question was asked as to why disposal scalpels were not in use. EHS is actively investigating this accident and providing assistance to evaluate and implement the use of a safer scalpel.

The second discussion focused on the recent exposure of an employee to a recombinant adenovirus when a pump system connection failed, spraying adenovirus into the employee's face. Pre-determined requirements for facility Personal Protective Equipment, pressure-monitoring devices, and secondary

containment were not adhered to, and serve as contributing factors to this exposure incident. This exposure is also reportable to the Office of Biotechnology Activities and will be reported by the Biological Safety Officer. The IBC will request a "cease and decist" order for the use of similar types of equipment in conjuction with infectious or potentially infectious material at this facility until the accident investigation can be completed and proposed revised procedures can be reviewed, observed, and approved.

The final accident discussion focused on the exposure of a student intern to a dead fox (road-killed) while performing necropsy for tissue collection. This work is not covered under an approved IBC registration and there is a potential for exposure to potentially infectious material and zoonotic pathogens (such as rabies). The IBC will request a "cease and decist" order on this work until an IBC document describing how this work can be done safely is reviewed and approved by the IBC and other NCI-Frederick management. Dr. McVicar requested a vote on this proposed action and made a motion to issue a letter requesting the above IBC registration documents and informing the Principal Investigator that this is not a currently authorized activity, Dr. Hughes and Creekmore seconded and all were in favor.

REVIEW OF PROTOCOLS

NEW IBC REGISTRATIONS

09-38 (Dr. O'Brien) *Human genetic analysis.*

-There are two issues regarding EBV: the handling of EBV itself (the virus) which would be addressed in Part C; and the EBV that is often present in cell lines with which you are working. Both of those are mentioned in this document but not sufficiently addressed. It was previously indicated that a separate IBC would come in for the EBV but both hazards are mentioned within this document, making the review a bit confusing.
-Provide assurance that materials/samples received into the lab for processing are inactivated prior to or at the time of receipt?
-Please clearly define the overall process from start to finish (from the time a sample is received and onward)?
-Are samples instantaneously inactivated? Are the samples used in the form of tissues, inactivated extracts of DNA, are the samples frozen? The understanding is that the LGD is going after immortalized cells, making DNA, making cell lines, and making PBL's. All of these processes need to be described and associated risks covered within the IBC document.
-There is a statement regarding "No pathogenic viruses are manipulated". If LGD is handling EBV and/or human cell lines this is not the case.

Dr. McVicar made a motion to defer approval, Dr. Creekmore seconded and all were in favor.

09-46 (Dr. Palmieri) *Development of a Mouse Model of Inflammatory Breast Cancer Brain Metastasis.*

-A1: SIM149 should be SUM 149
-A6: this answer should be "YES" given that you also answered A6a and A6a1.
-Please answer A6b.
-Part D: D5 should be "YES" instead of "NO"
-Human pathogen screening tests will be required for all human cell lines going into animals.
-D9a: Clarify what a "screen top centrifuge tube is and is a containment centrifuge used with this container?
-When vortexing will be performed, will this be done inside of a BSC and how will the potential for the creation of aerosols be mitigated?
-E8a: This answer should be modified as the human cell lines could be potentially hazardous and should be treated as "other potentially infectious material" given their potential to harbor human pathogens.
-E9: Human cell lines are not deemed as "certified free of infectious virus" and should be handled as potentially infectious material at all times. Although the pathogen screen results may all be negative, this is not a comprehensive test in scope.

Ms. Bell made a motion to conditionally approve, Dr. Garfinkel seconded and all were in favor.

09-49 (Dr. Hatfield) *Knockout mice missing the selenophosphate synthetase 1 (SPS1) gene.*

There were no comments or questions for this document.

Dr. Kozlov made a motion to approve, Dr. Freed seconded and all were in favor.

09-50 (Dr. Natarajan) *Laboratory of Molecular Cell Biology/CSP-Influenza Projects.*

-The samples are identified as nasal swabs. What will be shipped (i.e. how will you receive these samples)? Will the nasal swab come themselves or will they be immersed in some type of media or will you only receive the liquid media sample?
-If swabs are received, how will they be handled safely? There is a potential for contamination on the outside of the sample container so a procedure would be necessary to state how these samples will be received, opened, handled and that the sample container is disinfected to avoid contamination of gloves, etc.

Dr. Hughes made a motion to approve, Dr. Morin seconded and all were in favor.

09-51 (Dr. Blumenthal/Puri) *Novel Lipid-Based Nanoparticles for Targeted and Triggered Drug Delivery.*

-Do these studies involve any rDNA work? Although Part B has not been completed, there is a question as to where the protein(s) come from to coat the particles? If these involve rDNA work, then Part B will need to be completed.
-D6: this answer should be yes since Raji and Ramos cells are known to contain EBV.
-D9: Please note that the centrifugation will occur only with sealed tubes and provide information as to other containment measures in place that will be used to prevent aerosols (i.e. rotor buckets and safety caps, aerosol ring seals, etc).

Dr. Derse made a motion to conditionally approve, Dr. Hughes seconded and all were in favor.

RENEWALS

09-44(Dr. McNeil) *NCL In vitro and In vivo Characterization Assays.*

-Clarify what research activities will be performed inside of a BSC.
-Identify all work activities that will occur on the open bench. All work with human blood or blood products, and the work with human cells that will be infected with murine viral vectors should be conducted inside of a BSC. If there are procedures that need to be conducted outside of the BSC, we need to know what those activities are and how any risk(s) will be minimized or mitigated.
-Provide a statement describing the sharps to be used and how risks with those sharps will be minimized. (Right now the registration simply states that sharps will be used infrequently). The use of glass and sharps should be minimized for human blood and vector infected rodent cells. In those cases in which human cells will be injected into animals, we need to know how well the cells are characterized (human pathogen screening is required if it has not already been completed), how and by whom the injections will be done (if this is done by LASP trained personnel that is sufficient so please state that if that is the case).
-Will any nanoparticles be injected into animals? If so, same questions apply as in number 3) above with respect to vectors and cell lines. Please provide a statement to clarify if any nanoparticles will be injected and provide some details as to how this will be done safely.
-When putting an MLV related (in this case MSC) vector into mice, this could potentially result in the production of a mixed stock of viruses, some of which can infect humans (due to mobilization and recombination), since mouse cells can harbor both amphotropic and xenotropic endogenous viruses. Please clarify this potential risk within the document.
-The injection of human cells into mice increases the probability that some or all of the mice will shed viruses that can infect humans. Though the associated risks are not large, they are also not zero. Are any of the human cells known to produce amphotropic or xenotropic viruses? Will NCL be isolating cells from the tumors

generated in the mice?
-Will nanoparticles be used in any of the work covered by this application? If so, the experiments involving nanoparticles needs to be described. Whenever there is a potential for combined risks (such as the use of nanoparticles with chemical or biological material), the potential hazards of each material independently, as well as their combined risks, should be identified and addressed as best as possible. If this is the case with respect to your research scope, please provide a statement that will address these potential concerns.
-B3 paragraph (4th sentence following sequences) says “this amendment”. I believe this is intended as a renewal document.
-B5f2: B5 is answered “YES” but B5f2 response “no viral particles” conflicts with this response. Please correct this.
-Similar to the preceding question, B5h also says “NO” but wouldn’t there be a potential to generate aerosols (mentioned in A3 response) when cells are virally transduced? Response to B5h1response should provide operating procedures implemented to minimize or mitigate the risks. You may also want to reference the SOP/GLP steps 6 and 14 here as well as the NCL safe work practices as they are pertinent here.
-B10a: Clarify which activities with cells will occur in a CFH and which activities the BSC will be used for.
-Clarify safety practices and procedures with respect to the vector mentioned in Part B, which is not directly addressed in the SOP.

Dr. Hughes made a motion to defer approval to lead reviewers, Dr. Creekmore seconded and all were in favor.

09-45 (Dr. Phillips) *Renewal of 06-19.*

-Please clarify the processes the staff members use to assess and mitigate the risks in their various incoming projects. For example: Is the actual identity of the incoming item known to the staff members handling it? If so: Has a Material Safety Data Sheet been obtained and reviewed for potential hazards to determine how the material will be handled?
-Does the laboratory handle items of unknown or uncertain identity?
-What assumptions are made in that case, to determine lab practices and procedures? For example, a recent re-testing of former incoming projects in some screening activities suggests an uncertainty or, retrospectively, false identity of about 30%.
-If a MSDS is not available and/or the material has unidentified hazards, how are these materials handled?
-Does your group list and consider the known toxicities (if any are already known)?
-Is an LD50 known or can one be estimated? If not, how do they handle this case?
-What risks are posed within the laboratory based on the procedures and equipment/processes used with the materials? (e.g. aerosol or dust exposures, trans-dermal penetration, etc.)

-How is it determined when an engineering control (such as a chemical fume hood or BSC is used) and what personal protective equipment or practices and procedures are necessary to perform the work safely? What engineering measures (fume hoods, BSC's, PPE's, respirators, etc) are in the laboratory and available to the staff? In Part D only a Chemical Fume Hood is indicated but not a Biological Safety Cabinet (BSC), although the work with clinical specimens would need to be performed within a BSC.
-How is the laboratory or individual work projects separated so that the several staff members can work on several projects at once with minimal risk of cross-contamination?
-Does your laboratory receive anything that may be infectious?
-In A1 if you describe your overall process in document this will make it more clear as to what material you receive and what you do with it.
-A laboratory standard operating procedure is needed to address handling of human material. (If you require guidance on how to write an SOP for this, please reference the attached document).
-Please address the hazards posed by using the red cross blood (it is our understanding that RC Blood is not screened).

Dr. Creekmore made a motion to defer approval, Dr. Baseler seconded and all were in favor.

09-47 (Dr. F. Ruscetti) *Mechanism of Transmission of Human Lymphotropic Virus (HTLV) and Xenotropic MuLV Related Virus (XMRV).*

-Section A1: "...use of other full-length viruses". If these include ONLY HIV-1, HTLV-1/2, MLV, and XMRV, then the statement is ok, as the registration deals with retroviruses. If they are using other viruses (e.g. VSV), then we need to know about it. Please further define the statement.
-Section A61a: Transport needs to be in a sealed, leakproof secondary container.
-Part B, MLV Ampho... Section B4 and B5f1: Ampho env (or XMRV env) used in combination with MLV vector systems run the likelihood of recombining to create an infectious virus. Please have statements to this effect included in the registration.
-Part B, on all versions: "No potential hazards" should be changed to minimal hazards. Most of the experiments list human cells and by definition are BSL-2 (e.g. Some level of hazard and not "no hazard").
-Section B6a, add the following statement to all Part B's: "All work with infectious or potentially infectious materials will be carried out in a BSC and/or containment centrifuge", provided this is a true statement.
-Part B, HIV (parts B and C): Section B5g1: '...it depends on the env..." That's correct, and the hazard would go way up if the env is from HIV. Please include a statement that indicates that "if HIV env is used in conjunction with HIV based vectors, then there could be the possiblity that infectious and possibly replication competent HIV would be generated — albeit with a low probability as the accessory

genes have been removed." (i.e. there will be no combination of HIV cores with envelopes).
-Provide a statement within a comprehensive laboratory SOP document covering what will be done in the event of a spill and an injury.
-Can you reiterate in each part B related to work with HTLV, XMRV, and HIV-1 that BSL-2* practices, procedures and facilities will be used, as mentioned in Part A 3.
-In A4a, can the pipettes mentioned here be disinfected with bleach prior to disposal?
-Clarify that volume specified in C11a for HIV-1 is 0.05 mls and not Liters?
-For experiments with the potential to generate infectious recombinants, will these also be conducted in a BSL-2*?
-Just as an FYI, vector virus bearing HIV-1, ampho-MLV, XMRV, HIV-1 Env or VSV-G can infect human cells but for only a single cycle.
-There are statements within the document saying that no virus will be generated, however, virus-binding assays will be performed.
-More specific questions and comments pertaining to each separate section are as follows-
-FOR HIV-1: B5e2 for HIV-1 (correct "lector" to "vector")
-This work should be done in a BSL-2* facility.
-For all answers, correct the word "hood" to biological safety cabinet(especially in B5h1 and B10a).
-In B6a, this will not eliminate the possibility of recombination (again, BSL-2* should be used).
-B6c: clarify this statement. These plasmids are infectious.
-B11: Indicate the use of a BSC.
-FOR Glucose transport: B8b1 should be "viral sequence" instead of "virus"
-FOR Viral ENV vectors-VSV-G: B5g should be "yes" and single round infection only specified.
-B5h1: Use of BSL-2* should be mentioned here.
-B5J: RG2 does not need to be checked.
-B11: Check BSC box.
-FOR Soluble HTLV ENV vector: B5a-small typo; change "ot" to "to"
-B5h1 addresses "infectious virus"—what infectious virus is being discussed here? And change "hood" to BSC.
-B6a: should say minimal hazards instead of "no potential hazards". Please list the minimal hazards and how those will be mitigated (i.e. through the use of a BSC, and BSL-2 practices per the SOP).
-B7b should be "NO"
-B10a: Correct hood.
-B11: indicate BSC.
-FOR HTLV ENV Vector: B5e4: should this say "pseudotype" instead of "generate"?
-B5g should be yes and specify single round infection cycle
-B5h1: correct "hood" and specify use of BSL-2*
-B10a: Correct hood.
-B11: indicate BSC.
-FOR HTLV Molecular clones: B4: correct spelling for transfected.

-B5a: correct 203-T cells to 293 cells.
-B5c1: missing part of a sentence after the word “portion”.
-B5e1, second paragraph: how can this be done without making virions (this response does not appear to coincide with the response below in B5g1 regarding the generation of “cell-free virus”.
-B5h1: correct “hood” and specify use of BSL-2*.
-B5j should be RG2 only.
-B7b is no.
-B10: correct “hood”
-FOR XMRV ENV Vectors: B5e4: change generate to “pseudotype”?
-B5g: should be yes and specify single round infection cycle
-B5h1: will BSL-2 or 2* be used for this work?
-B10a: Correct hood.
-B11: indicate BSC.
-FOR Infectious HTLV: C9a: will this be done in a BSL-2 or 2*?
-C12a1 and C13: correct “hood”
-FOR Part D, primary cells from XMRV patients: D9a: correct “hood” and specify BSL-2 or 2*
-FOR ampho ENV expression vectors: B5g: should be “yes” for and specify single round infection cycle
-B5h1: Specify BSL-2 or 2*
-B6a: says “no potential hazards” but what about potential for recombination to generate replication competent amphotropic material?
-B10a: Correct hood.
-B11: indicate BSC
-FOR Neuropilin expression vectors: B5h1: correct “hood”
-B8b1: change “virus present” to “viral sequence”
-B10a: correct “hood”
-FOR Syndecan expression vectors: B5g1: change “virus present” to “viral sequence”
-B6a: B6a: should say minimal hazards instead of “no potential hazards”. Please list the minimal hazards and how those will be mitigated (i.e. through the use of a BSC, and BSL-2 practices per the SOP).
-B10a: Correct hood

Dr. McVicar made a motion to defer approval to the lead reviewers, Ms. Bell seconded and all were in favor.

09-48 (Dr. Salcedo) *Role of Inflammation in Carcinogenesis*

-Please, confirm that no viral work will be used in the frame of this IBC. B5 is answered “NO” but B5a – B5j is completed.
-Please clarify what the three human cancer lines (listed in D3a) will be used for and where this work will be done. Will these cells be injected in mice? Will the cells be genetically manipulated in vitro, and if yes, how (transfection, infection, …)? If

human cell lines will be injected into mice, human pathogen screening will be required if not already completed.
-Please, confirm that animal dosing/applications with be done only by trained LASP personnel who is clearly instructed to the toxic and/or carcinogenic nature of administered reagents and potential risks.
-Please answer B2c (mice).
-For centrifugation, please state what safety measures are put into place (i.e. containment centrifuge, aerosol ring, rotor buckets with safety caps, etc) to avoid spills/aerosols.

Dr. Kozlov made a motion to approve, Dr. Derse seconded and all were in favor.

AMENDMENTS

06-109 (Dr. Bustin) – pending PI submitting amendment request.

06-76 (Dr. Hughes/Rein) – pending edits for clarification.

06-102 (Dr. Kuehn) – pending PI submitting amendment request.

All other amendments submitted at this time have been approved.

OUTSTANDING ITEMS

Updates were provided to the Committee by Ms. Bell.

OTHER BUSINESS

BBP Compliance: 98%

Human Pathogen Screen Update:
-The human pathogen screening should transition from AHDL to MGD sometime Fall 2009. Updates will be forthcoming.

The meeting was adjourned at 1:52 p.m.

_____ _____
Theresa D. Bell, MPH, CBSP Date
IBC Secretary
Biological Safety Officer, EHS

APPROVED:

_____ _____
Dan McVicar, Ph.D. Date
Chairman, NCI-Frederick IBC

xc: Dr. Reynolds
Mr. Wheatley
Dr. Arthur
Mr. Bufter