UofU Department of Public Safety

Case Report for Incident 17-0646

Nature: Animal Problem
Location: HSC

Address: 15 N East Medical Dr; EEJMRB
Salt Lake City UT 84108

Offense Codes: INFO
Received By: Lewis, M
How Received: T
Agency: UUPD
Responding Officers: James, R
Responsible Officer: James, R
Disposition: CLO 04/18/17
When Reported: 18:40:54 04/18/17
Occurred Between: 18:40:39 04/18/17 and 18:40:39 04/18/17

Assigned To:
Detail:
Date Assigned: **/**/**
Status:
Status Date: **/**/**
Due Date: **/**/**

Complainant: 38626
Last: [redacted]
First: [redacted]
Mid:
DOB: [redacted]
Dr Lic: [redacted]
Address:
Race:
Sex: [redacted]
Phone: [redacted]
City: [redacted]

Offense Codes

Reported: NC Not Classified
Observed:
Additional Offense: INFO Information

Circumstances

Responding Officers:
James, R

Unit :
P58

Responsible Officer: James, R
Received By: Lewis, M
How Received: T Telephone
When Reported: 18:40:54 04/18/17
Judicial Status: CAMP
Misc Entry:

Agency: UUPD
Last Radio Log: 19:13:33 04/18/17 CMPLT
Clearance: CMP Completed
Disposition: CLO **Date:** 04/18/17
Occurred between: 18:40:39 04/18/17
and: 18:40:39 04/18/17

Modus Operandi:
Description :
Method :

Involvements

Date	Type	Description	
04/18/17	Name	[redacted]	Information

04/18/17	Name	[redacted]	Complainant
04/18/17	Name		Victim
04/18/17	Cad Call	18:40:54 04/18/17 Animal Problem	Initiating Call

_____

Responsible LEO:

_____

Approved by:

_____

Date

Supplement

17-0646
R James 58
Tue Apr 18 21:24:34 MDT 2017

On Tue Apr 18 18:40:00 MDT 2017 I was dispatched to the [redacted] for an animal problem.

Upon arrival I met with the victim who stated he was bit by a mouse. The victim stated his supervisor requested a Police report.

NOTHING FOLLOWS

R JAMES 58

Tue Apr 18 21:26:30 MDT 2017

Supplement

17-0646
R James 58
Tue Apr 18 21:26:54 MDT 2017

On Tue Apr 18 18:40:00 MDT 2017 I was dispatched to the [redacted] for an animal problem.

Upon arrival I met with [redacted], a research assistant. [redacted] stated he received a bite from an experimental mouse while working on Mon Apr 16 20:00:00 MDT 2017. [redacted] stated the mouse bit him on the second finger of his right hand. A photo of the bite mark has been attached to this report.

[redacted] stated as per protocol he reported the bite to the OU Office and scheduled a medical appointment for Wed Apr 19 10:30:00 MDT 2017.

I informed [redacted] that I am more than happy to do a report for him but it is not necessary for workman's compensation. [redacted] stated his supervisor, [redacted], requested he file a report with Police.

[redacted] stated he did not need medical attention.

EHS has been notified.

NOTHING FOLLOWS

R JAMES 58

Tue Apr 18 21:36:49 MDT 2017

Name Involvements:

Information : 27458

Last: [redacted] **First:** [redacted] **Mid:**

DOB: **Dr Lic:** **Address:** [redacted]

Race: [redacted] **Sex:** [redacted] **Phone:** [redacted] **City:** [redacted]

Victim : 38626

Last: [redacted] **First:** [redacted] **Mid:**

DOB: **Dr Lic:** **Address:** [redacted]

Race: **Sex:** [redacted] **Phone:** [redacted] **City:** [redacted]

UNIVERSITY OF UTAH POLICE

1735 E. South Campus Dr. Salt Lake City, Utah 801-585-2577

WITNESS STATEMENT

NAME [redacted] 38626 DATE OF BIRTH: [redacted] MM/DD/YY

ADDRESS: [redacted] CITY [redacted] STATE: [redacted] ZIP: [redacted]

PHONE: (Home/Cell) _____ (Work) [redacted]

TYPE OF INCIDENT: Assault (ie. Theft, Accident, Burglary, Assault, ect.) LOCATION: [redacted]

DATE OF INCIDENT: 4/17/17 TIME: 2000 AM/PM

Please read the following before filling out your statement:

Pursuant to Rule 1102, Utah Rules of Evidence and Section 76-8-504, Utah Code Annotated: You are hereby notified that the statements you are about to make in this document may be presented to a magistrate or a judge in lieu of your sworn testimony at a preliminary examination. **Any false statement you make and that you do not believe to be true may be subject to criminal punishment as a Class B misdemeanor.**

Please describe below what you saw, heard or know about this incident:

(Use back of form if more space is needed)

Unfortunately I was bit by my experimental mouse yesterday night in [redacted] My second finger of right hand was bleeding and I reported it to my supervisor [redacted] According the injury protocol, I reported it to the UU office at the [redacted] I made an appointment with [redacted] @10:30 to 4/18/2017

Signature (required) [redacted] Date 4/18/2017

INVESTIGATING OFFICER R James 58 Case # 17-0646

Incident Number: 17-0646

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AAALAC Accreditation (Health Sciences): 17 FEB 2016
AAALAC Accreditation (College of Science): 25 MAR 2015
PHS Assurance Registration Number: A3031-01
USDA Registration Number: 87-R-0001

Principal Investigator: [redacted]
Protocol Number: 17-04018
Protocol Title: A Novel Antibody Formulation for Cancer Treatment
Date Protocol Received: 20 APR 2017
Date of Approval: 22 MAY 2017
Date of Expiration: 21 MAY 2020

Protocol Summary:

The prognosis for advanced cancers remains poor because of lack of effective therapies. The US FDA has approved a variety of anticancer antibodies (Ab); however, these Abs have limited anti-cancer activity. We have recently developed a novel strategy to formulate the Abs, which substantially enhances Abs' anti-cancer activity. Further preclinical studies in animal models are needed to determine therapeutic capacity of the Ab formulation.

Your animal protocol was reviewed at a convened IACUC meeting, completed by Designated Member Review and approved on the date listed above.

Please be aware that serious or repeated adverse events (e.g., a large number of postoperative complications, excessive or unexpected mortality rate) must be reported timely to the IACUC committee. The notification should include a brief summary of the adverse event and any corrective actions. It is further required to report if any of the adverse events lead to a change in pain categories (e.g., unalleviated pain or severe distress, category E).

For your convenience a copy of your approved protocol is enclosed.

[redacted] 5/22/17

IACUC Date

cc: [redacted]

IACUC
Phone: [redacted]
Email: [redacted]

IACUC Assigned Protocol Number: 17-04018

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Research Protocol with Animals

Failure to follow instructions completely and using specified format may result in return of application (Refer to http://iacuc.utah.edu, guideline number: IACUC G001).

Section A

A 1.0 Principal Investigator: [redacted] U of U Title/Rank: [redacted]

Department: [redacted] Office Phone: [redacted]

Campus Address: [redacted] Cell Phone: [redacted]

U of U E-mail: [redacted]

Laboratory Contact: [redacted] Phone: [redacted]

E-mail: [redacted]

Emergency Contact: [redacted] Daytime phone: [redacted]

Phone (After hours): [redacted]

A 2.0 Project Title: A novel antibody formulation for cancer treatment

Please do not use acronyms or abbreviations in the title of the protocol.

A 2.1 Provide a brief project hypothesis (one short paragraph).

The prognosis for advanced cancers remains poor because of lack of effective therapies. The US FDA has approved a variety of anticancer antibodies (Ab); however, these Abs have limited anti-cancer activity. We have recently developed a novel strategy to formulate the Abs, which substantially enhances Abs' anti-cancer activity. Further preclinical studies in animal models are needed to determine therapeutic capacity of the Ab formulation.

A 3.0 Specify time period covered by this protocol:

☒ 3 Years from date of IACUC approval

Does this replace a previous protocol? No: ☐ Yes*: ☒ Previous protocol #: 14-05013

*Be aware that the protocol being replaced will be early terminated.

Will animals be transferred from the previous protocol? ☐ No Yes ☒ How many?* 80

If yes, clarify what has been done to them: About 30 mice are transplated with human tumor and receiving treatment

**These are animals that are alive and on study and found housed on campus. For rodent colonies, provide approximate numbers of animals. However, the PI must maintain accurate records to assure that unapproved animals procedures are not used in this research protocol.*

A 4.0 Funding sources: list sources, grant number and U of U account number (must be listed or pending).

a. Department of [redacted]

b. [redacted]

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Was the funding source(s) for animal work to be done on this protocol supported by a scientific peer review panel?
If the work is not federally funded then two individuals, of your choice, must peer review your work.

☒ No Yes ☐

If funding is not supported by a peer review panel, provide names of two peer reviewers (both reviewers must have already read the application and are not involved in the study)

a. [redacted]
b. [redacted]

Title of Grant (if different from this application):

The same

Will funding be administered by U of U Office of Sponsored Projects? ☐ Yes ☒ No

If no, clarify who: [redacted]

A 5.0 Procedures to be performed (Check as many as appropriate):

☒ Tissue harvest at necropsy (tissue harvest at euthanasia is not considered surgery)
☒ Post-procedural monitoring is required (see C 5.4 for definition)
☒ Breeding colony required
☒ Tumor production is required

A 6.0 Location where study related activities occur

Location where animals will be housed

Building # or name	Room #
[redacted]	[redacted]

*"Tab" to add additional lines

Laboratory where study procedures will be performed

Building # or name	Room #
[redacted]	[redacted]

*"Tab" to add additional lines

Section B

B 1.0 Euthanasia

Any changes to the method of euthanasia determined in consultation with the clinical veterinarian MUST be reported to the IACUC. A protocol amendment is necessary to initiate continued change in the method of euthanasia from the original protocol.

Adoption or Euthanasia Methods (All methods must conform to the recommendations published by the AVMA Panel on Euthanasia unless approved by the IACUC) Any animal may be released for adoption with institutional veterinarian approval.

B 1.1 Location where euthanasia will be performed

Building # or name	Room #
[redacted]	[redacted]

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The IACUC approved application may be modified by the IACUC to remove non-essential sections that do not pertain to the submitted research

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B 1.2 Inhalant agents (isoflurane, CO_2*, other)[1]

Animal Species: Rodents Agent: CO2. - a CO2 tank is located in room 1250 in EEJ.

[1] *A secondary physical method is recommended to confirm death* Policy *CMC (Comparative Medicine Center) to use standard operating procedure TS017, Euthanasia of Animals. *CO_2 - the dose not required to be listed (CO_2 must come from compressed gas and not dry ice)*

If a secondary physical method will not be used, provide rationale and indicate how death will be confirmed (i.e., evaluation of vital signs):
Death will be confirmed by cessation of breathing over 5 minutes.

I have read and agreed to follow the IACUC Policy *P010, Euthanasia of Laboratory Animals*
☒ Yes (http:// Euthanasia of Laboratory Animals)

B 3.0 Tumor Studies

B 3.1 If transplantable tumors or injected tumor cells will be used, have they tested negative for murine pathogens? (The results must be submitted to [redacted]

Yes: ☒ No: ☐

B 3.2 Indicate the source of the cells: American Type of Cell Culture (ATCC)

B 3.3 Injection procedure related only to tumor production:

Species	Tumor/cells	Route	Site	Volume	# of tumors per animal
Mice	Raji	iv	Blood	200 ul	1
Mice	BxPC3	subcutaneous	Flank	100 ul	1

I have read and agreed to follow the IACUC Policy *P007, Solid Tumor Production*
☒ Yes (http:// Solid Tumor Production)

B 4.0 Blood Samples

B 4.1 Route: Submandibular Amount: 50 ul Frequency: Once a month Total Volume: Maximum amount: 150 ul

B 4.3 Describe the blood collection procedure (include time between blood draws): - Submandibular sinus will be punctured with a lancet and about 50 ul will be collected into a Epndorff tube. Mice will be observed for 30' to ensure wellbeing

B 5.0 Hazardous Agents

If the research requires the use of potentially hazardous agent(s) (i.e. pathogenic agents, carcinogens, toxic chemicals, radioisotopes, and rDNA), specify the agent(s) to be used and indicate that Occupational and Environmental Health and Safety (EHS), Institutional Biosafety Committee (IBC), Radiological Health (RAD) or consultation with Occupational Health Medicine at the University of Utah has been notified and agents registered.

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Hazardous Agent(s): Clearance pending with Environmental Health and Safety regarding the use of human tumor cells, human lymphocytes and chemotherapeutics

B 5.1 Occupational and Environmental Health and Safety ([redacted]) - toxic chemicals, carcinogens, and other toxins.

Occupational and Environmental Health Dept. registration http://ehs.utah.edu/research-safety/iacuc/:

Occupational and Environmental Health Dept. registrations: N/A: ☐

Registration date: Clearance pending with Environmental Health and Safety regarding the use of chemotherapeutics

Describe the required animal husbandry practices approved by OEHS, how animal cages will be identified, and how OCM staff will be notified:

Clearance pending with Environmental Health and Safety regarding the use of chemotherapeutics.

B 5.3 Institutional Biosafety Committee ([redacted]) - viruses, bacteria, parasites, recombinant DNA, transgene, human cells.

Institutional Biosafety Approval N/A: ☐

Date of approval: Clearance pending with IBC regarding the use of human tumor cells and human lymphocytes IBC Submission #:

Biosafety Level (BSL) of the agent EH&S Biosafety Manual BSL level:

Describe the required animal husbandry practices approved by IBC, how animal cages will be identified, and how OCM staff will be notified:

Pending with IBC

B 8.0 All animals are housed and cared for according to the *Guide for the Care and Use of Laboratory Animals* and the *Animal Welfare Act Regulations and Standards*. This includes but is not limited to: the animal's living conditions, housing, feeding, and non-medical care. All animal care is directed by a veterinarian. Medical care is available when necessary and is provided by a qualified veterinarian.

Yes: ☒ No: ☐

B 8.3 Single housing of social species should be the exception. Justify social animal species being housed individually (i.e., mice, rats, dogs, cats, non-human primates, rabbits, guinea pigs, swine, goats, sheep, cows). When necessary, single housing of social animals should be limited to the minimum period necessary and, where possible, visual, auditory, olfactory and, depending on the species, protected tactile contact with compatible conspecifics should be provided.

Is this species a social animal? ☒ **Yes** ☐ **No**

B 8.3.1 If a social species will be single housed, provide justification. This includes single housing of animals after a procedure, during treatment, dosing, and/or as a result of being the last animal on study.

Describe: - Single female mouse will be integrated with other female mice; male mouse will be housed with a suitable mate. If a suitable mate is not available, the mouse will be euthanized

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Expected length of time animals may be single housed due to experiment: 3 days

B 8.3.2 If a breeding colony is maintained, please indicate any circumstances that may require animals to be single housed.

☒ Pregnant female mice may be separated from males before giving birth. The male mouse will remain single housed until the pair is rebred (3-4 weeks) to avoid fighting by breeder males. In trios, pregnant females are moved to individual cages prior to delivery to avoid multiple litters in one cage (3-4 weeks).

☒ Male mice weaned from a given litter are housed together. However, if there is only one male from the litter, the animal is housed singly or until a suitable mate is identified, as adding older males is likely to cause fighting (breeding age is 7-8 weeks, could be housed singly for 4-5 weeks). When a litter is weaned and there is only one female, she will be housed alone, until other females of the same genotype are identified (1-2 weeks) or until a mate is identified (4-5 weeks).

B 12.0 Animal Transport

Transportation of animals in private vehicles is discouraged because of potential animal biosecurity, safety, health, and liability risks for the animals, personnel, and institution. A transportation service provided by the [redacted] is available and is encouraged to be used. Please contact [redacted] for additional information and/or to schedule transport.

Will [redacted] staff be responsible for transporting animals?

☒ Yes ☐ No

Section C

C 1.0 Explain the scientific merit of the proposal.

Describe: B-cell non-Hodgkin's lymphoma (NHL) is one of the most common cancers in the US, and treatment is associated with substantial healthcare costs. The FDA has approved several anti-CD20 antibodies (Ab) for NHL treatment, however these Abs are not cytotoxic to tumor cells and thus have limited therapeutic capacities. Currently, low-grade NHLs are incurable and many high-grade NHLs relapse or are refractory to therapy. Treatment of refractory/relapsed NHL is often very expensive with serious toxicity, complications and therapeutic failure. While there has been continuous search for more effective anti-CD20 Abs, the newer Abs still appear to have limited activity and are used in combination with cytotoxic drugs in induction therapies. Making anti-CD20 Ab cytotoxic to NHL should improve treatment outcome and reduce expense. It is established that crosslinking CD20 by multivalent Abs or Ab-toxin conjugates confer direct cytotoxicity for NHL cells. Graphene oxide (GO) is a unique, one-atom thick 2-dimentional carbon-based nanomaterial (nanosheet) with enormous (per unit) and extremely flexible planar surface that can stably bind a large number of peptides or protein molecules through noncovalent binds. GO at high concentrations causes oxidative stress and lipid membrane damage, and can kill bacteria and cancer cells. We hypothesize that association of anti-CD20 Ab with GO will result in multivalent Ab/GO complexes capable of killing NHL cells through crosslinking CD20 and induction of oxidative stress. We have demonstrated that the anti-CD20 Abs such as rituximab (RTX) and GO form stable, multivalent complexes (RTX/GO) that are highly cytotoxic to all the tested NHL cell lines. When administered intravenously into mice, RTX/GO eliminates established xenograft systemic Burkitt's lymphoma through necrosis, whereas free RTX fails to do so.

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Reconstitution of lymphoma-bearing mice with human lymphocytes or association of IFN with RTX/GO further enhances RTX/GO therapeutic capacity. This application will test the ability of RTX/GO to cure refractory lymphomas in the absence of toxic therapies in preclinical animal models. In We will compare RTX/GO with the newest FDA-approved anti-CD20 Ab obinutuzumab (OBN) for their activity against aggressive and refractory lymphomas. As OBN is non-cytotoxic, established chemotherapy drugs will be used in combination with OBN to achieve killing of resistant NHL cells. The nonspecific toxicity of the OBN/drug combination vs RTX/GO will be examined *in vitro* and *in vivo* in human lymphocyte-reconstituted lymphoma-bearing mouse models. We hypothesize that RTX/GO will specifically eliminate resistant lymphomas without causing side effects or affecting T or NK cells, while OBN relying on high-dose chemotherapy to kill the lymphoma cells will cause serious toxicity to the hosts and damage the reconstituting human lymphocytes. In ***Aim 2***: We will test strategies to further enhance therapeutic efficacy of RTX/GO by RTX/GO-mediated targeted delivery of immune cytokines to the sites of lymphoma in order to stimulate antitumor immunity, which is likely essential to achieving cure in patients. While the tumor-destructive, lymphocytes-sparing feature of RTX/GO therapy provides a unique opportunity for antitumor immunity to occur, the intratumor tolerogenic microenvironment can allow NHL to evade immunity. Immune cytokines such as IL-12 and IFN are proven to be highly effective in breaking the tolerogenic mechanisms, but their clinical application has been precluded by the serious toxic side effects associated with systemic administration of adequate dose. Targeted cytokine delivery through RTX/GO should circumvent this barrier. The impact of systemic vs targeted delivery of cytokines will be compared in the presence or absence of human lymphocytes. Together, these studies should establish a novel, low-cost, nontoxic and effective therapy for refractory NHL and accelerate commercialization of the technology for clinical application.

The success with RTX prompted us to investigate on another FDA-approved Ab, trastuzumab (TRA) which is specific for the oncogenic receptor HER2 (1). An increasing variety of cancer types are now found to overexpress HER2 (2-13), including osteosarcoma (OS) (14) and pancreatic ductal adenocarcinoma (PDA) (4). However, FDA has only approved TRA for treatment of HER2$^+$ breast and gastroesophageal cancers because therapeutic impact of TRA on other cancer types is yet unknown. Indeed, clinical trials identified no therapeutic benefit of TRA on HER2$^+$ OS or PDA (4, 15, 16). Our results showed that the anti-OS capacity of TRA was substantially enhanced by associating TRA with GO, and GO-associated (TRA/GO) but not free TRA elicited oxidative stress and intensive detrimental signaling through HER2, leading to necroptosis of the target OS cells. Intravenous administration of TRA/GO into the immunodeficient NRG mice bearing xenograft human OS eradicated the established OS (Fig. 1a&b), resulting in indefinite survival of the animals (Fig. 1c). No side effect was observed of TRA/GO treatment. While our results demonstrated promising activity of GO-associated Abs against lymphoma and sarcoma, it is yet unclear whether the antibody formulation would be similarly effective against the most commonly diagnosed cancers, carcinoma, the malignancies of epithelial origin. Establishment of effectiveness on carcinoma should have both important clinical and commercialization implications. We propose to use PDA as a model carcinoma to test our formulation as PDA is one of the most malignant cancers defying all current treatment with extremely poor prognosis (17). HER2 overexpression is reported in 45% PDA (18). Although previous studies including clinical trials identified no anti-PDA activity of TRA (4, 16, 19), we have found in preliminary study that TRA/GO bind to a PDA cell line BxPC3 with much stronger reactivity than free TRA. We propose to further test TRA/GO on a panel of available PDA cell lines (19) to determine the general activity of the formulation against various HER2$^+$ PDAs. The TRA/GO formulation will also be tested *in vivo* in xenograft human PDA mouse models to determine their therapeutic efficacy as described in our previous studies (20). **Alternative approaches:** We anticipate potent anti-PDA activity of TRA/GO similarly with OS. Should unexpected results occur that TRA/GO was not as effective, especially on PDA cells expressing very low levels of HER2, additional Abs such as anti-HER3 or anti-EGFR would be used in combination with TRA to generate HER2+HER3/GO or HER2+EGFR/GO. Previous studies have reported enhanced anti-PDA activity with dual antibody targeting (21, 22). **Another alternative**

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approach would involve activation of the host antitumor immunity, which is now known to play an important role in controlling cancer progression (23, 24). Intratumoral tolerogenic microenvironment constitutes an important mechanism to suppress antitumor immunity. HER2-targeted delivery of immune cytokines to the tumor site might disrupt such mechanism (25). In a recent clinical trial, we have reported that IFNα, when used in combination with targeted therapy induces strong CD8 T-cell mediated tumor immunity, leading to durable remission of advanced sarcomas (26). IL-12 has also emerged as one of the most potent cytokines in overcoming tumor-induced tolerance to activate antitumor immunity in many studies (27). However, systemic administration of large doses of cytokines often causes serious life-threatening side effects, precluding their application in patients. Targeted cytokine delivery to tumors should circumvent this important barrier. In preliminary study, we found that pre-incubating 20 μg of GO with 18×10^5 IU of IFNα (about 1 μg of protein) before incubation with 200 μg of RTX allowed stable association of both the cytokine and Ab with GO. We will take similar approach for targeted delivery of IFNα or IL12 to PDA through TRA GO. We anticipate that formulation of cytokine TRA GO would kill target PDA cells and at the same time deliver the cytokines to the necrotic tumor to activate antitumor immunity. The source of the immune cells in our mouse model will be derived from adoptively transferred human lymphocytes. Accomplishment of this milestone should establish a preclinical proof of principle for the therapeutic efficacy of TRA GO against PDA, which, if successful, would make this technology highly attractive in a large market of cancer therapeutics.

C 2.0 Rationale for the number of animals being requested.

C 2.1 Species, column designation, and total number of animals requested for project period (this includes the approximate number of live animals to be transferred, in-house, from previous protocol)

All animals are to be included in the table in this section. This includes experimental, donor, training, breeding pairs, pregnant mothers, and offspring that cannot be utilized because of genotype, sex, etc.

Animal Species	Column B # Animals	Column C # Animals	Column D # Animals	Column E # Animals
Mice	36		144 - 192	

Column B = Breeding colony; Column C = non-painful procedures; Column D = Procedures with alleviated pain; Column E = Painful procedure

The procedures to be performed are tail vein injection and subcutaneous inoculation of the cells. To alleviated pain, the injection will be done using insulin syringes which is not expected to introduce significant pain, and the injection will be completed within a few seconds. Therefore, no additional pain alleviation approaches should be needed.

C 2.2 Describe the breeding colony. This should include a rationale for the approximate number of animals needed for breeding pairs, how many pups the breeding pair are assumed to foster, how the breeding colony is structured, what strains are being maintained, and what percentages of pups will be unsuitable for study due to genetic differences. The colony design should show how the total number of study animals (total number of animals in column B, C, D, and E) will be generated.

Six breading cages consisting of 18 NRG mice (one male plus two females per cage) of and six breading cages containing 18 NSG mice will be set to generate 180 pups (6-8 pups from each mother). 144 - 192 pups the breeding pair are assumed to foster. The breeding colony is structured with one male plus two females per cage. Breeding animals will be replaced once every 9 months. The NRG and NSG strains are being maintained. 0% of pups will be unsuitable for study due to genetic differences.

Column B: 18 NRG + 18 NSG = 36
Column D: 12 NRG female x 6-8 pups = 12 NSG female x 6-8 pups = 144 - 192

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Statistical analyses will be performed on the experiment results to determine if differences observed between the treated groups and control groups statistically significant. If the trend indicates a difference but the statistical analyses does not support, additional number of animals might be need to added to the study.

C 2.3 Describe the number of animals required for the experiments proposed in this study, including the number of study groups, time points, and the number of animals in each test and control group (does not include breeding colony). For ease of understanding the research, a table showing animals and study groups is highly encouraged.

Describe: Each cancer cell line will be engrafted subcutaneously to 40 mice iv or subcutaneously at the inguinal area. The tumor bearing mice will be divided into 8 groups (5 mice each), with 4 groups given lymphocytes while the other 4 groups not, and each group to receive the either following treatment. Tumor cell subcutaneous inoculation and tail vein injection of the treatments will be performed without anesthesia.

Without lymphocytes
1) PBS control (5 mice)
2) RTX + gemcitabine + IFNα (5 mice)
3) Nano particle (GO) (5 mice)
4) Cetuximab-GO-gencitabin-IFNα (5 mice)

With lymphocytes
5) PBS control (5 mice)
6) RTX + gemcitabine + IFNα (5 mice)
7) Nano particle (GO) (5 mice)
8) RTX-GO-IFNα (5 mice)

Thus, 5 x 8 = 40 mice are needed with one cancer cell line. If the results are consistent, generating data with statistical significant, the experiment will be completed. If not, experiment may need repeated with additional 40 mice. Therefore, total of 160 mice might be needed for two cancer models. The treatment will be given intravenously in 200 ul volume through the tail vein every other day. Total of 5 treatments will be given in 10 days. The tumor size will be measured twice a week, and the mice will be sacrificed whenever the tumors grow to 1.5 cm in diameter or their body weights drop by 20%. The mice will also closely be followed for signs of discomfort including hair ruffling, hunched back, reduced activity, and weight loss. Treatment will be held if the sign of discomfort is identified due to treatment.

C 2.3.1 Provide justification for the number of animals per group.

Statistical power analysis (and/or),
Student T test will be used to compare the tumor size between the treatment groups. As variations invariably occur in experiments, a few mice are needed in each group. If the real difference in tumor size resulting from different treatments, there will be a good chance that 5 mice per group give rise to a statistically significant p value of < 0.05 in statistical analysis.

Literature reference that supports the number of animals per group (and/or),

As it is difficult to calculate p values without actual data, two references are now provided below. The references used similar tumor cell lines DLD1 and BxPC3 and minimal number of 5 to 10 mice per group in their studies

Lee J, Lee I, Han B, Park JO, Jang J, Park C, Kang WK **Effect of simvastatin on cetuximab resistance in human colorectal cancer with KRAS mutations.** J Natl Cancer Inst. 2011 Apr 20;103(8):674-88. doi: 10.1093/jnci/djr070. Epub 2011 Mar 11

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Larbouret C, Robert B, Bascoul-Mollevi C, Penault-Llorca F, Ho-Pun-Cheung A, Morisseau S, Navarro-Teulon I, Mach JP, Pèlegrin A, Azria D. **Combined cetuximab and trastuzumab are superior to gemcitabine in the treatment of human pancreatic carcinoma xenografts.** Ann Oncol. 2010 Jan;21(1):98-103. doi: 10.1093/annonc/mdp496. Epub 2009 Nov 4.

C 2.4 The numbers provided in Sections C 2.2 and 2.3 matches the numbers provided for each category in the table in Section C 2.1.
Yes: ☒ No: ☐

C 3.0 Rationale for the use of animals and the species being used.

Provide description of the choice of animal species selected. Why were they chosen (each species)? What makes them unique to other animal models?

We will perform a variety of cell culture experiments to obtain all the important information on the effectiveness of the proposed therapy. However, since the ultimate purpose of this study is to use the threapeutics to treat cancers in patients, the most relevant, reliable information can only be collected in live animal models at preclinical stages. Mice are a superior model system for this study because they are small animals that breed rapidly. Many features of the immune system are share between mousse and human. We propose to use two well-studied human lymphoma cell lines as well as carcinoma cell lines BxPC3 (pancreatic, Kras WT) and DLD1 (colon, Kras mutated) to generate tumor models in immune-deficient NOD/rag r-/- mice that will be reconstituted with human lymphocytes, and test the capacity of the antibody-coupled gemcitabine and IFNα in eliminating the two cancers. In this approach, gemcitabine and IFNα are linked to antibody Cetuximab that recognizes EGFR expressed on the tumor cells, we therefore hypothesize that the drug would be effectively delivered directly to the tumor, reducing the possibility to damage the normal tissue, including the lymphocytes. On the other hand, the high concentration of IFNα delivered to the tumor site would activate antigen presenting cells and lymphocytes, resulting in antitumor immunity. While many aspect of this hypothesis will be test in cell culture, the therapeutic outcome needs to be examined in mouse models.

C 5.0 Provide a clear and concise sequential description of the study groups and what the animal(s) will experience while on the study. Include all procedures and tests (recordings, test and control groups, etc.).

Describe: Each cancer cell line will be engrafted into to 40 mice either iv or subcutaneously at the inguinal area. The tumor bearing mice will be divided into 8 groups (5 mice each), with 4 groups given lymphocytes while the other 4 groups not, and each group to receive the either following treatment. Tumor cell subcutaneous inoculation and tail vein injection of the treatments will be performed without anesthesia. Tumor will grow at the sites of inoculation, and mice will be either transplant human lymphocytes. These are not expected to cause significant pain. The mice will be divided into 8 groups and treated as indicated below

Without lymphocytes
1) PBS control (5 mice)
2) RTX/TRA + gemcitabine + IFNα (5 mice)
3) Nano particle (GO) (5 mice)
4) RTX/TRA-GO-gencitabin-IFNα (5 mice)

With lymphocytes: 2x10*7 human lymphocytes in 200 ul will be administered ip.

5) PBS control (5 mice)
6) RTX/TRA + gemcitabine + IFNα (5 mice)

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7) Nano particle (GO) (5 mice)
8) RTX TRA-GO-IFNα (5 mice)

Dose: PBS 200 ul; GO 4 ug 25g; RTX 20 ug 25g; ; TRA 20 ug 25g; ; CTX 20 ug 25g, 0.06 ug 25g; gemcitabine 0.5 mg 25g.

Time to start treatment: Treatment will begin tumor grow to 40 mm^3 or two week of iv transplantation.

Blood collected will be for low cytometry

The mice will be closely evaluated on daily basis for signs of discomfort including hair ruffling, hunched back, reduced activity, and weight loss. The animals will be scarified if body weights drop by 20%. The tumor size will be measured twice a week, and the mice will be sacrificed whenever the tumors grow to 1.5 cm in diameter. It is very unlikely that the treatments with rituximab, cetuximab, trastuzumab or the antibody GO formulation, or IFNα should induce significant discomfort to the animals. The antibodies and IFNα are FDA-approved for use in patients, and the corresponding target are absent on mouse cells. GO is proven to nontoxic to mice in previous studies. However, the chemotherapeutics might cause discomfort distress, which are observed with patients receiving chemotherapy, such as loss of appetite, nausea and vomiting, and weight loss. To reduce the possibility and degree of distress, we will use the minimum effective dose published in previous studies, such as with gemcitabine at 200 mg kg given at 11 hours of circadian times after light onset (HALO). Studies have found that this gemcitabine dosing produces least body weight loss and least neutropenia after injection at 11, whether the drug was given alone or with cisplatin (P=0.001) (28). In case that mice develop signs of discomfort due to the treatments, the treatment will be held or discontinued

1. Li XM, Tanaka K, Sun J, Filipski E, Kayitalire L, Focan C, et al. Preclinical relevance of dosing time for the therapeutic index of gemcitabine-cisplatin. Br J Cancer. 2005;92(9):1684-9.

C 5.1 Describe any non-surgical procedures conducted under anesthesia (MRI, injections, x-ray). This should include the frequency of imaging.

Describe: Tumor cell subcutaneous inoculation and tail vein injection of the tumor cells and treatments will be performed without anesthesia.

C 5.2 Describe the potential or possible impact of the procedures (this includes surgery and/or study procedures) on the animals' well-being.

Describe: It is very unlikely that the treatments with rituximab, cetuximab, trastuzumab or the antibody GO formulation, or IFNα should induce significant discomfort to the animals. The antibodies and IFNα are FDA-approved for use in patients, and the corresponding target are absent on mouse cells. GO is proven to nontoxic to mice in previous studies. However, the chemotherapeutics might cause discomfort distress, which are observed with patients receiving chemotherapy, such as loss of appetite, nausea and vomiting, and weight loss. To reduce the possibility and degree of distress, we will use the minimum effective dose published in previous studies, such as with gemcitabine at 200 mg kg given at 11 hours of circadian times after light onset (HALO). Studies have found that this gemcitabine dosing produces least body weight loss and least neutropenia after injection at 11, whether the drug was given alone or with cisplatin (P=0.001) (28). In case that mice develop signs of discomfort due to the treatments, the treatment will be held or discontinued

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1. Li XM, Tanaka K, Sun J, Filipski E, Kayitahire L, Focan C, et al. Preclinical relevance of dosing time for the therapeutic index of gemcitabine-cisplatin. Br J Cancer. 2005;92(9):1684-9.

C 5.2.1 **Describe any modified animal husbandry or animal care procedures for animals whose well-being is affected by the study procedures (e.g., food provided on cage floor, supplements provided, frequent bedding changes, etc.).**

In rare events that mice appear weak to reach food, semiliquid food will be provided. If the animals unexpectedly develop signs of discomfort due to the tumor growth metastasis or treatments as described above, the treatment will be held or discontinued, and mice scarified if body weights drop by 20%.

C 5.2.2 **Indicate the endpoint(s) in time when animals are scheduled to be euthanized for each study group (e.g., hours/days or/weeks).**

Experiment/study group	Study time point(s) for euthanasia
Raji, lymphoma	Paralysis, more than 20% weight loss, or on 100th day
BxPC3	Subcutaneous tumor > 1.5 cm, or on 100th day.

* *"Tab" to add additional lines*

☒ I have also read and will comply with the IACUC policy *P003, Death as an Endpoint*.

C 5.3 **Describe conditions when animals may need to be terminated earlier than the expected endpoint due to expected or unexpected study results or circumstances. What are the specific signs/symptoms that will prompt early euthanasia? (*Moribund animals can be euthanized without the Principal Investigator's consent if Institutional Veterinarian or his her designee dictates; the health evaluations of animals in quarantine and not on study are under the direction of a veterinarian). Potential endpoints: moribund, loss of weight exceeding 20% of normal body weight, inappetence, hunched posture, ruffled fur, etc. See IACUC Guideline G013, Pain, Distress, and Humane Endpoints http://Gd13 Pain, Distress, and Humane Endpoints.*

Describe: At early stages, the animal's health and well-being will not be affected by the lymphoma. At late stage, lymphoma will cause paralysis or weight loss. When this occurs or weight loss reaches 20% of the body weight, the animal will be euthanized.
The mice will also be closely followed for signs of discomfort including hair ruffling, hunched back, reduced activity, and weight loss, and will be euthanized whenever their body weights drop by 20%. The mice will also be euthanized whenever the tumors grow to 1.5 cm in diameter.

C 5.4 **Describe Post-procedural Monitoring – Observations and/or treatments that are required as a result of the procedure(s) conducted on an animal(s). This includes primarily surgical, non-surgical procedures, and/or drug/chemical procedures, but may also include induced behavioral changes. Most often, the monitoring consists of the monitoring of vital signs, administration of analgesics, antibiotics, parenteral fluids, or other drugs. These activities are recorded in the animal's health record. This should also include any general health monitoring parameters required after research procedures. This would also include tumor burden, toxicity, and/or disease progression monitoring. However, this does not include monitoring of the animals during recovery from anesthesia (this is to be described in sections C 4.4.9 and/or C 5.1.5).**

Describe: The tumor size will be measured and mice weighed twice a week. The mice will be sacrificed whenever the tumors grow to 1.5 cm in diameter. The mice will also be closely followed for signs of discomfort including hair ruffling, hunched back, reduced activity, and weight loss, and scarified if body weights drop by 20%. It is very unlikely that the treatments should induce discomfort to the mice due to side effect because RTX, TRA, gemcitabine, and IFNα are all FDA-approved medicine for use in patients. GO is

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also proven to nontoxic to mice in previous studies. In addition, all the above reagents will be used at lower doses as compared to previous studies because of anticipated high anti-cancer potency of the targeted delivery of the drugs. In unexpected events that mice develop signs of discomfort due to the treatments as described above, the treatment will be held or discontinued.

In addition to the general daily observations made by the [redacted] staff:

Indicate the frequency of study related post-procedure monitoring: Check daily; tumor measured twice a week, and weight obtained twice a week.

Indicate the duration of study related pos-procedure monitoring (e.g., duration of study, first 3 post-operative days, etc.): 100 days

C 5.4.1 Indicate who is responsible for post-procedural monitoring and administration of medications.

[redacted]

C 6.0 Identify all procedures in this protocol application that may cause more than momentary pain or distress to the animals. This would include surgical procedures even if the animal is under anesthesia and/or administered pain relief medication.

Describe: The procedure performed will include s.c. tumor cell inoculation, tail vein injection. These are not expected to cause significant pain.

C 6.2 Describe any of the expected symptoms that might become painful (e.g., toxicity, tumor size, disease progression, etc.) and indicate when animals will be euthanized for humane reasons.

After inoculation of 5 x 10*6 of tumor cells subcutaneously, the tumor will become palpable in about 10 days, and grow to 1 cm in diameter in about 3-4 week. The tumor growth is not expected to cause pain or distress to the mice according the previous studies including those cited above, as the mice normally eat, act and grow normally, and no analgesias were used in previous studies. It is therefore unlikely the tumor growth should cause significant pain in our experiment. Nevertheless, the tumor size will be measured and mice weighed twice a week. The mice will also be closely followed for signs of discomfort including hair ruffling, hunched back, reduced activity, and weight loss, and scarified if body weights drop by 20%. The mice will also be sacrificed whenever the tumors grow to 1.5 cm in diameter, which is also the endpoint.

It is also very unlikely that the treatments should induce discomfort to the mice due to side effect of the medicine because trastuzumab, gemcitabine, and IFNα are all FDA-approved medicine for use in patients. GO is also proven to nontoxic to mice in previous studies. In addition, all the above reagents will be used at lower doses as compared to previous studies because of anticipated high anti-cancer potency of the targeted delivery of the drugs. In unexpected events that mice develop signs of discomfort due to the treatments as described above, the treatment will be held or discontinued.

C 6.3.1 If no analgesics are given, and animals will be in potential pain or distress, scientifically justify. Please provide full literature reference. (This must be indicated for the use of both opioids and NSAID's)

Lee J1, Lee I, Han B, Park JO, Jang J, Park C, Kang WK Effect of simvastatin on cetuximab resistance in human colorectal cancer with KRAS mutations. J Natl Cancer Inst. 2011 Apr 20;103(8):674-88. doi: 10.1093/jnci/djr070. Epub 2011 Mar 11.

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Larbouret C, Robert B, Bascoul-Mollevi C, Penault-Llorca F, Ho-Pun-Cheung A, Morisseau S, Navarro-Teulon I, Mach JP, Pelegrin A, Azria D. Combined cetuximab and trastuzumab are superior to gemcitabine in the treatment of human pancreatic carcinoma xenografts. Ann Oncol. 2010 Jan;21(1):98-103. doi: 10.1093/annonc/mdp496. Epub 2009 Nov 4.

C 7.0 Personnel involved in the study. Please answer the following:

C 7.2 List All Other Personnel Working on this Study (*tab to expand table*).

Name	Responsibility* (I, S, A, P, E, O)	Describe experience, hands-on training, and/or other responsibilities. If no previous experience/training, who will train the individual?
[redacted]	I, P, E, O	Extensive prior experience plus further training with PI and staff member at [redacted].
[redacted]	I, P, E, O	Extensive prior experience plus further training with PI and staff member at [redacted].

*Responsibility: I = Injections/blood collection S = Surgery A = Anesthesia P = Post-procedure monitoring E = Euthanasia O = Other animal procedures

C 7.3 Have all individuals that come in contact with the animals (and the Principal Investigator) in the project completed the online species-specific, University of Utah Core, Common Compliance Issues, and Occupational Health training modules provided by the U of U Institutional Trainer? Yes: ☒ No: ☐

C 8.0 Alternatives (literature search). Must contain the following (for help contact Training Coordinator [redacted]

C 8.1 Database selection (Choose those that are appropriate for the area of this study. The use of one database is seldom adequate. Some examples are: Medline, Agricola, Embase, CAB, BIOSIS):

List databases used (at least two): Medline, Agricola

C 8.2 Date of search: 4-4-2017

C 8.3 Years of search (must be a minimum of 5 years): 5

C 8.4 Key words used in the search (Must use the words: pain, distress, alternative, anesthesia, analgesia, and non-animal model as well as any key words/concepts using terminology from your protocol). List all words used in the search.

List all words used: Raji, 4RH, Bx-PC3, Gemcitabine, IFNa, grapheme oxide (GO), pain, distress, alternative, anesthesia, analgesia, and non-animal model

C 8.5 Describe in detail the search strategies. Be sure to put the word "or", "and/or" between the key words (see instructions for help G001 http://iacuc.utah.edu - Search instructions):
Describe Strategy:

1) Raji 4RH OR Bx-PC3 AND mice AND pain.
2) Gemcitabine OR IFNa OR grapheme oxide (GO) AND mice AND distress
3) Raji 4RH OR Bx-PC3 AND analgesia
4) Raji 4RH OR Bx-PC3 OR Gemcitabine OR IFNa OR grapheme oxide (GO) AND immunity AND non-animal model
5) Gemcitabine AND IFNa AND grapheme oxide (GO)

C 8.6 Provide a written description to demonstrate how the following were considered.

C 8.6.1 Replacement of animal models with alternative models (such as computer or in-vitro models).

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The IACUC approved application may be modified by the IACUC to remove non-essential sections that do not pertain to the submitted research

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In search for non-animal model system to study the propose regimen on treatment of the two human cancers, literature search was performed using "Raji 4RH OR Bx-PC3 OR Gemcitabine OR IFNa OR grapheme oxide (GO) AND immunity AND non-animal model". No results were yield. This is expected as the reagents included in the proposal are new, and experiment designed are novel. In addition, only animals have immune system similar to the humans.

C 8.6.2 Refinement of the experimental design and/or procedural techniques (such as modified techniques, housing modifications, and modified restraint).

To learn alternative experimental design concerning pain induced by the tumor, or distress resulted from the proposed treatment, literature search was performed using the following.
a) "Raji 4RH OR Bx-PC3 AND mice AND pain"
b) "Raji 4RH OR Bx-PC3 AND analgesia"
c) "Gemcitabine OR IFNa OR grapheme oxide (GO) AND mice AND distress"
No result was obtained. It is consistent with general observation the mice bearing subcutaneous tumors do not manifest signs of pain or distress as they act, eat and grow normally.
It is very unlikely that the treatments should induce discomfort to the mice due to side effect because cetuximab, gemcitabine, and IFNα are all FDA-approved medicine for use in patients. GO is also proven to nontoxic to mice in previous studies. In addition, all the above reagents will be used at lower doses as compared to previous studies because of anticipated high anti-cancer potency of the targeted delivery of the drugs.

C 8.6.3 Reduction of the number of animals used.

In most similar studies, 5 to 15 mice in each group are used and experiment are usually repeated once or twice. In the current proposal, only minimal number of 5 mice in each group are planned to use. It is the PI's intention not to repeat the experiment if the results are statistically convincing.

C 8.7 Provide a written assurance that experiments described in this protocol do not unnecessarily duplicate previous experiments.

Search using "Gemcitabine AND IFNa AND grapheme oxide (GO) yield no results, confirming that the proposed study is novel and not previously performed.

C 8.8 Provide a written assurance that alternatives to painful procedures (found in this research) have been considered and no alternative methods have been found.

Literature search was performed using the following.
a) "Raji 4RH OR Bx-PC3 AND mice AND pain"
b) "Raji 4RH OR Bx-PC3 AND analgesia"
c) "Gemcitabine OR IFNa OR grapheme oxide (GO) AND mice AND distress"

No result was obtained. It is consistent with general observation the mice bearing subcutaneous tumors do not manifest signs of pain or distress as they act, eat and grow normally.

Section D **Principal Investigator Assurance (Each item in section D must be checked or provide explanation).* Note: Any unresolved regulatory deficiencies found during the conduct of this study may be reported to the PI's department Dean and Chair by the IACUC.**

☒ D 1.0 Any adverse event (unexpected results in animal health and welfare) must be reported to the IACUC.

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IACUC Assigned Protocol Number: 17-04018

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☒	D 2.0	The experiments described in this protocol do not unnecessarily duplicate previous experiments.
☒	D 3.0	No animal will be used in more than one major operative procedure from which it is allowed to recover without scientific justification and approval from the IACUC.
☒	D 4.0	Paralytics will not be used without appropriate anesthesia and proper monitoring.
☒	D 5.0	Alternative methods for conducting these procedures have been thoroughly reviewed.
☒	D 6.0	Animals obtained under this protocol may be used only as described herein (including approved amendments).
☒	D 7.0	Written amendment must be approved by the IACUC if the protocol should require revision or changes (i.e. a change in procedures, different animal species, additional animals, different investigator, additional technicians, etc.).
☒	D 8.0	This protocol is an exact representation of any proposed animal work being submitted to federal funding agencies and the funding agencies are listed in this application.
☒	D 9.0	In the event that animals are on study when this protocol expires, or is suspended by the IACUC, and/or excess animals on study, they will be automatically transferred to a veterinary husbandry protocol and are under the direction of the University of Utah institutional veterinarian. All animal husbandry expenses continue to be the responsibility of the Principal Investigator of the expired or suspended protocol until the animals are no longer held under the husbandry protocol.
☒	D 10.0	Veterinary consultation must and will occur when pain or distress is beyond the level anticipated in the protocol description or when interventional control is not possible.
☒	D 11.0	This protocol will be immediately expired by the IACUC office if a replacement protocol is submitted by the Principal Investigator and approved by the IACUC.
☒	D 12.0	The Principal Investigator will make available an approved protocol to their research staff listed on the protocol and assure that they are knowledgeable as to its contents.

For IACUC office use only

[redacted]

5/22/17

Date of approval

Veterinarian assigned: [redacted]

Comments: FCR - DMR

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April 27, 2017

[redacted]
University of Utah
Campus

Subject: Protocol Clarification 17-04018
Title: A Novel Antibody Formulation for Cancer Treatment

Dear [redacted]:

The above mentioned protocol was reviewed at the last IACUC meeting, which was held on April 26, 2017. The committee members have some additional questions and ask that you respond as quickly as possible. Final disposition will take place once your response has been received.

Please respond to the following:

- Do not send in a revised protocol application. Only send in answers to the questions. The committee only wants to see the answers to the committee questions. If you submit a revised protocol in addition to the requested answers, the revised protocol will not be processed.

1. Please provide the IBC approval letter for the use of human tumor cells in this research project.
2. Please provide the OEH&S approval for the use of chemotherapeutics in this research project.
3. The committee determined that the 129 study animals should be placed in category C (non-painful procedure). Please indicate if this is acceptable.

If you have any questions please call our office.

Sincerely,
IACUC Office
[redacted]

IACUC
Phone: [redacted]
Email: [redacted]

For IACUC office use only: **APPROVED**

[redacted] 6/6/17

IACUC Signature Date of review

Note: [illegible]

Animal Transfer Form

NOTE: Complete all sections. Only one animal species can be transferred on this form. Send completed form to [redacted]

A 1.0 Today's Date 06-05-2017

A 2.0 Protocol **FROM** which animals will be transferred: 17-04018

A 2.1 Name of Principal Investigator to send animals: [redacted]

A 3.0 Protocol number **TO** which animals will be transferred: 17-03006

A 3.1 Name of Principal Investigator to receive animals: [redacted]

A 4.0 Number of animals to be transferred: 4

A 5.0 Animal Species: Mus Musculus

A 6.0 Describe what research has been done on the animals being transferred (if any).

Describe: None

A 7.0 Describe why the animals should be transferred (what is the reason for the transfer).

Describe: Breeding

***NOTE!**

Please be aware that the principal investigator of the receiving protocol is responsible to ensure that the use of the transferred animal(s) is in accordance with the approved protocol (including approved amendments). An additional protocol amendment would be required to request any protocol changes and/or new research.

[redacted] 6/6/17

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Occupational and Environmental Health and Safety
125 South Fort Douglas Blvd, Bldg 605 SLC, UT 84113 (801)581-6590

To: [redacted] Cc: [redacted]

From: [redacted]

Date: 05.15.2017

Subject: Review of Hazardous Chemical Use in IACUC Protocol #17-04018

On May 4, 2017 [redacted] submitted an Application for the use of Hazardous Chemicals in Animal Protocol #17-04018 to Occupational and Environmental Health and Safety (OEHS). [redacted] is identified as the Principal Investigator. This application has been reviewed and the use of the hazardous chemicals as outlined in the application has been approved.

The chemicals approved for use are as follows:

Gemcitabine

This approval will remain valid for a period of three (3) years from the date of this letter. A new application will need to be submitted for approval at that time if continued use of the hazardous material identified in the indicated protocol will be required.

Please contact me with any questions or requests for additional information.

[redacted]

From: [redacted]
Sent: Monday, May 22, 2017 2:36 PM
To: [redacted]
Subject: FW: FW: Clarification for Protocol 17-04018

[redacted] Please process. [redacted]

From: [redacted]
Sent: Monday, May 22, 2017 2:29 PM
To: [redacted]
Cc: [redacted]
Subject: Re: FW: Clarification for Protocol 17-04018

[redacted]

We have provisionally approved his protocol at ABSL1.

[redacted]

On Tuesday, May 16, 2017, 10:27:54 AM MDT, [redacted] wrote:

HI [redacted]

Our office had a question for [redacted] that he was to ask your dept. It is in the attached Pdf. Is it ok for us to approve is protocol? Are you ok with him using human tumor cells?

[redacted]

IACUC Director

From: [redacted]
Sent: Thursday, April 27, 2017 11:33 AM
To: [redacted]
Subject: Clarification for Protocol 17-04018

[redacted]

Attached is a clarification letter for protocol 17-04018.

Sincerely,

[redacted]

Training Coordinator

IACUC- University of Utah

[redacted]

(Please use the iacuc email (Link) to provide the department with comments and suggestions concerning office procedures and services.)

THE UNIVERSITY OF UTAH

PI: [redacted]
University of Utah
Campus

Prescreen date: April 6, 2017
Subject: Protocol prescreen: 17-04018
Title: A Novel Antibody Formulation for Cancer Treatment

The protocol application listed above has been assigned a U of U IACUC protocol number (also listed above). Please use this assigned protocol application number with any correspondence to our office. A prescreen has been conducted on the protocol application and some procedures need to be clarified. Please respond to these questions in letter format stating the question and then the answer.

These questions should be answered prior to the protocol being reviewed by the full IACUC membership. This information if received by the IACUC office by April 13, 2017 will be included in the next committee review meeting.

Please provide the following information:

- Send in a revised protocol application. Include the answers to the questions listed below in the revised protocol. The revised protocol must be received for the committee to review your application. The revised protocol must be reviewed and approved by the clinical veterinarian. Any revised application received without veterinary review by the assigned veterinarian will not be accepted.

- For ease of the committee to again audit this protocol, and make sure that questions were answered, it is required that you provide a separate document answering the below questions (restate the question and provide the answers; one by one). Separate answers must be received for the committee to consider your application complete.

1. Section A, 4.0, Funding
    a. Please indicate the funding source(s) for this research project.
    b. If the work is not federally funded, please indicate the names of the two peer reviewers and provide assurance that they agree with the study design.

2. Section A, 5.0, Procedures to be performed.
    a. Please be aware that post-procedural monitoring should have been checked. Please clarify.
    b. Please be aware that special husbandry conditions are required should have been checked since animals will be fed a special diet. Please clarify.
    c. The protocol application indicates that gas anesthetics will be required on this study (e.g., Isoflurane) used in a vaporizer or bell jar. Please clarify as the use of a gas anesthetic is not described.
    d. Please be aware that tissue harvest at necropsy should have been checked since tissue will be harvested. Please clarify.

3. Section B, 1.1, Location where euthanasia will be performed.
    a. Please clarify if a CO_2 tank is located in room 1250 in EEJ.

THE UNIVERSITY OF UTAH

4. Section B, 1.2, Inhalant agents for euthanasia.
    a. If a secondary physical method will not be used to ensure death, provide rationale and indicate how death will be confirmed (i.e., evaluation of vital signs).

5. Section B, 3.4, Tumor studies.
    a. The check box to indicate that you have read and agree to follow IACUC Policy P007, Solid Tumor Production was not marked. Please clarify and provide assurance that you have read and agree to follow P007, Solid Tumor Production.

6. Section B, 4.0, Blood samples.
    a. Please clarify the maximum amount of blood that may be drawn from each animal.

7. Section B, 4.3, Describe the blood collection procedure.
    a. Please provide a description of the blood collection procedure.

8. Section B, 5.0, Hazardous Agents.
    a. It would seem that the use of human tumor cells and human lymphocytes should be listed in this section of the application. Please clarify.
    b. Please indicate if the Blood Borne Pathogen training has been completed by the laboratory personnel for the use of human tumor cells.
    c. It would also seem that the use of the indicated chemotherapeutics should be listed in this section of the application. Please clarify.

9. Section B, 5.1, Occupational and Environmental Health and Safety ([redacted] – toxic chemicals, carcinogens, and other toxins.
    a. Please clarify if Occupational and Environmental Health and Safety approval is required for the use of chemotherapeutic agents in this research project.
    b. Please describe the required animal husbandry practices approved by OEHS, how animal cages will be identified, and how OCM staff will be notified.

10. Section B, 5.3 Institutional Biosafety Committee ([redacted] - viruses, bacteria, parasites, rDNA, transgene, human cells.
    a. Please clarify if IBC approval is required for the use of human tumor cells and human lymphocytes.
    b. Please describe the required animal husbandry practices approved by IBC, how animal cages will be identified, and how OCM staff will be notified.

11. Section B, 8.3, Single housing of social species (dogs, cats, non-human primates, rabbits, mice, rats, guinea pigs, sheep, swine, goats, calves).
    a. Please clarify if there may be any circumstances in which an animal may be single housed. This includes single housing of animals after a procedure, during treatment, dosing, last animal on study, etc.

12. Section C, 2.1, Animal designation.
    a. The protocol application lists the study animals in category D (procedures with alleviated pain). Please clarify the painful procedure in which pain will be alleviated.

13. Section C, 2.1, Total number of animals requested.
    a. The protocol application lists 18 animals in category B and 160 animals in category D. However, the protocol indicates in section A 3.0 that 200 animals will be transferred from the previous protocol. The total number of animals listed in the table should also include the animals to be transferred from the previous protocol. Please clarify.

b. The protocol application indicates that "About 80 - 100% pups will be used." Please clarify and indicate if the unused pups are included in the total number of animals listed in the table in this section of the application. If not, these pups should be listed in category C.

14. Section C, 2.2, Describe the breeding colony.
    a. Breeding colony. Please further describe the breeding colony. This should include a rationale for the total number of animals needed for breeding pairs, how many pups the breeding pair are assumed to foster, how the breeding colony is structured, what strains are being maintained, and how many pups will be unsuitable for study due to genetic differences. The overall design of the breeding colony should be to support the number of animals required for testing. The colony should show how the study animals will be generated.
    b. Please indicate how often the breeding animals will be replaced.

15. Section C, 2.3, Describe the number of animals required for this study.
    a. The protocol application indicates in this section "If the results are consistent, generating data with statistical significant, the experiment will be completed. If not, experiment may need repeated with additional 40 mice." Please provide additional information to further clarify/justify this.

16. Section C, 5.0, Provide a clear and concise sequential description of what the animal(s) will experience while on study. Include all procedures and tests (recordings, test and control groups, etc.).
    a. Please provide additional information to further describe each study group. A clear and concise sequential description of the study groups and what the animal(s) will experience while on study is required.
    b. Please indicate the dose of each treatment to be administered.
    c. The protocol application indicates in section A 6.0 that irradiation will be performed in EEJ. However, irradiation is not described. Please clarify and/or provide a complete description of this part of the study.
    d. Please clarify which animals will undergo blood collection and clarify what analyses will be performed on the collected blood.
    e. Please indicate the dose/volume of lymphocytes to be administered and the route of administration.
    f. The protocol application indicates in this section that "Tumor cell subcutaneous inoculation and tail vein injection of the treatments will be performed without anesthesia." Please clarify if the animals will be anesthetized for the IV injection of tumor cells. If so, please complete section C 5.1-5.1.5 in the revised protocol.
    g. Please indicate when in relation to tumor development will the treatment begin.
    h. The protocol application indicates in section C 1.0 that "We also propose to use TUBO cells, a mouse carcinoma cell line expressing human HER2 (29) to establish a congenic HER2+ mouse carcinoma model to further characterize therapeutic function of cytokine-associated TRA/GO in a immunocompetent host." However, this does not appear to be described in the application. Please clarify and/or provide a complete description of this part of the study.

17. Section C, 5.2.2, Indicate the endpoints(s) in time when animals are scheduled to be euthanized for each study group (i.e., hours/days/ or weeks).
    a. The box to indicate that no experiments are performed on live animals, only euthanasia and tissue harvest was checked. Please clarify.

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18. Section C, 6.2. Describe any of the expected symptoms that might become painful (i.e., toxicity, tumor size, disease progression) and indicate when animals will be euthanized for humane reasons.
    a. Please further describe the tumor progression (lymphoma) and its effects on the animal's health and well-being.
    b. If applicable, please describe the potential effects of irradiation on the animal's health and well-being and indicate end points that may be used to early remove an animal from study.

19. Section C, 7.2, List all other personnel working on the study.
    a. The protocol application indicates that the listed personnel will be responsible for anesthesia (A) and surgery (S). However, anesthesia and surgery do not appear to be a part of this study. Please clarify and/or correct this section in the revised protocol.

20. Attending veterinarian has helped plan the activities of this protocol. Our standard operating procedure for protocol development requires that the protocol be developed with the assistance of the U of U veterinarian.
    a. The protocol application has been assigned to [redacted] The protocol application and the protocol prescreen will be sent to him. Please be sure to work directly with the veterinarian to complete this requirement.
    b. Please provide assurance that the revised protocol, with changes listed above, has been reviewed and approved by [redacted]

THE UNIVERSITY OF UTAH

PI: [redacted]
University of Utah
Campus

Prescreen date: April 6, 2017
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Title: A Novel Antibody Formulation for Cancer Treatment

The protocol application listed above has been assigned a U of U IACUC protocol number (also listed above). Please use this assigned protocol application number with any correspondence to our office. A prescreen has been conducted on the protocol application and some procedures need to be clarified. Please respond to these questions in letter format stating the question and then the answer.

These questions should be answered prior to the protocol being reviewed by the full IACUC membership. This information if received by the IACUC office by April 13, 2017 will be included in the next committee review meeting.

Please provide the following information:

- Send in a revised protocol application. Include the answers to the questions listed below in the revised protocol. The revised protocol must be received for the committee to review your application. The revised protocol must be reviewed and approved by the clinical veterinarian. Any revised application received without veterinary review by the assigned veterinarian will not be accepted.

- For ease of the committee to again audit this protocol, and make sure that questions were answered, it is required that you provide a separate document answering the below questions (restate the question and provide the answers; one by one). Separate answers must be received for the committee to consider your application complete.

1. Section A, 4.0, Funding
   a. Please indicate the funding source(s) for this research project.
      - Department of [redacted]
   b. If the work is not federally funded, please indicate the names of the two peer reviewers and provide assurance that they agree with the study design.
      - [redacted]

2. Section A, 5.0, Procedures to be performed.
   a. Please be aware that post-procedural monitoring should have been checked. Please clarify.
   b. Please be aware that special husbandry conditions are required should have been checked since animals will be fed a special diet. Please clarify.
      ¶ Now checked.
   c. The protocol application indicates that gas anesthetics will be required on this study (e.g., Isoflurane) used in a vaporizer or bell jar. Please clarify as the use of a gas anesthetic is not described.
      - Gas anesthetics is now unchecked.
   d. Please be aware that tissue harvest at necropsy should have been checked since tissue will be harvested. Please clarify.

THE UNIVERSITY OF UTAH

- Now checked.

3. Section B, 1.1, Location where euthanasia will be performed.
   a. Please clarify if a CO_2 tank is located in room 1250 in EEJ.
      - Yes, a CO_2 tank is located in room 1250 in EEJ.

4. Section B, 1.2, Inhalant agents for euthanasia.
   a. If a secondary physical method will not be used to ensure death, provide rationale and indicate how death will be confirmed (i.e., evaluation of vital signs).
      - Death will be confirmed by cessation of breathing over 5 minutes.

5. Section B, 3.4, Tumor studies.
   a. The check box to indicate that you have read and agree to follow IACUC Policy P007, Solid Tumor Production was not marked. Please clarify and provide assurance that you have read and agree to follow P007, Solid Tumor Production.
      - Now checked, and I you have read and agree to follow P007, Solid Tumor Production.

6. Section B, 4.0, Blood samples.
   a. Please clarify the maximum amount of blood that may be drawn from each animal.
      - Maximum amount: 150 ul

7. Section B, 4.3, Describe the blood collection procedure.
   a. Please provide a description of the blood collection procedure.
      - Submandibular sinus will be punctured with a lancet and about 50 ul will be collected into a Epndorff tube. Mice will be observed for 30' afterwards to ensure wellbeing.

8. Section B, 5.0, Hazardous Agents.
   a. It would seem that the use of human tumor cells and human lymphocytes should be listed in this section of the application. Please clarify.
      - Clearance pending with Environmental Health and Safety regarding the use of human tumor cells and human lymphocytes.
   b. Please indicate if the Blood Borne Pathogen training has been completed by the laboratory personnel for the use of human tumor cells.
      - Clearance pending with Environmental Health and Safety regarding the use of human tumor cells and human lymphocytes.
      -
   c. It would also seem that the use of the indicated chemotherapeutics should be listed in this section of the application. Please clarify.
      - Clearance pending with Environmental Health and Safety regarding the use of chemotherapeutics.

9. Section B, 5.1, Occupational and Environmental Health and Safety ([redacted] – toxic chemicals, carcinogens, and other toxins.
   a. Please clarify if Occupational and Environmental Health and Safety approval is required for the use of chemotherapeutic agents in this research project.
      - Clearance pending with Environmental Health and Safety regarding the use of chemotherapeutics.

   b. Please describe the required animal husbandry practices approved by OEHS, how animal cages will be identified, and how [redacted] staff will be notified.

- Clearance pending with Environmental Health and Safety regarding the use of chemotherapeutics.
-

10. Section B, 5.3 Institutional Biosafety Committee ([redacted]) - viruses, bacteria, parasites, rDNA, transgene, human cells.
    a. Please clarify if IBC approval is required for the use of human tumor cells and human lymphocytes.
        - Clearance pending with IBC regarding the use of human tumor cells and human lymphocytes
    b. .
    c. Please describe the required animal husbandry practices approved by IBC, how animal cages will be identified, and how [redacted] staff will be notified.
    -Pending with IBC.

11. Section B, 8.3, Single housing of social species (dogs, cats, non-human primates, rabbits, mice, rats, guinea pigs, sheep, swine, goats, calves).
    a. Please clarify if there may be any circumstances in which an animal may be single housed. This includes single housing of animals after a procedure, during treatment, dosing, last animal on study, etc.
        - Single female mouse will be integrated with other female mice; male mouse will be housed with a suitable mate. If a suitable mate is not available, the mouse will be euthanized.

12. Section C, 2.1, Animal designation.
    a. The protocol application lists the study animals in category D (procedures with alleviated pain). Please clarify the painful procedure in which pain will be alleviated.
        - The procedures to be performed are tail vein injection and subcutaneous inoculation of the cells. To alleviated pain, the injection will be done using insulin syringes which is not expected to introduce significant pain, and the injection will be completed within a few seconds. Therefore, no additional pain alleviation approaches should be needed.

13. Section C, 2.1, Total number of animals requested.
    a. The protocol application lists 18 animals in category B and 160 animals in category D. However, the protocol indicates in section A 3.0 that 200 animals will be transferred from the previous protocol. The total number of animals listed in the table should also include the animals to be transferred from the previous protocol. Please clarify.
        - The protocol application now lists 36 animals in category B and 180 animals in category D. The protocol now indicates in section A 3.0 that 80 animals will be transferred from the previous protocol.
    b. The protocol application indicates that "About 80 -100% pups will be used." Please clarify and indicate if the unused pups are included in the total number of animals listed in the table in this section of the application. If not, these pups should be listed in category C.
        - It is likely that all the pups will be used. The unused pups are included in the total number of animals listed in the table in this section of the application.

14. Section C, 2.2, Describe the breeding colony.
    a. Breeding colony. Please further describe the breeding colony. This should include a rationale for the total number of animals needed for breeding pairs, how many pups the breeding pair are assumed to foster, how the breeding colony is structured, what strains

are being maintained, and how many pups will be unsuitable for study due to genetic differences. The overall design of the breeding colony should be to support the number of animals required for testing. The colony should show how the study animals will be generated.

- Six breading cages consisting of 18 NRG mice (one male plus two females per cage) of and six breading cages containing 18 NSG mice will be set to generate 180 pups (6-8 pups from each mother). 144 – 192 pups the breeding pair are assumed to foster. The breeding colony is structured with one male plus two females per cage. Breeding animals will be replaced once every 9 months. The NRG and NSG strains are being maintained. 0% of pups will be unsuitable for study due to genetic differences.

Column B: 18 NRG + 18 NSG = 36
Column D: 12 NRG female x 6-8 pups + 12 NSG female x 6-8 pups = 144 - 192

-

b. Please indicate how often the breeding animals will be replaced.
   - Breeding animals will be replaced once every 9 months.

15. Section C, 2.3, Describe the number of animals required for this study.
    a. The protocol application indicates in this section "If the results are consistent, generating data with statistical significant, the experiment will be completed. If not, experiment may need repeated with additional 40 mice." Please provide additional information to further clarify/justify this.

- Statistical analyses will be performed on the experiment results to determine if differences observed between the treated groups and control groups statistically significant. If the trend indicates a difference but the statistical analyses does not support, additional number of animals might be need to added to the study.

-

16. Section C, 5.0, Provide a clear and concise sequential description of what the animal(s) will experience while on study. Include all procedures and tests (recordings, test and control groups, etc.).
    a. Please provide additional information to further describe each study group. A clear and concise sequential description of the study groups and what the animal(s) will experience while on study is required.
       - Tumor will grow at the sites of inoculation, and mice will be either transplant human lymphocytes. These are not expected to cause significant pain. The mice will be divided into 8 groups and treated as indicated below:

       -
    b. Please indicate the dose of each treatment to be administered.
       - PBS 200 ul; GO 4 ug/25g; RTX 20 ug/25g; gemcitabine 0.5 mg/25g

       -
    c. The protocol application indicates in section A 6.0 that irradiation will be performed in EEJ. However, irradiation is not described. Please clarify and/or provide a complete description of this part of the study.

- No irradiation will be performed

-

d. Please clarify which animals will undergo blood collection and clarify what analyses will be performed on the collected blood.

   - Flow cytometry

e. Please indicate the dose/volume of lymphocytes to be administered and the route of administration.

   - 2x10*7 in 200 ul will be administered ip.
   -

f. The protocol application indicates in this section that "Tumor cell subcutaneous inoculation and tail vein injection of the treatments will be performed without anesthesia." Please clarify if the animals will be anesthetized for the IV injection of tumor cells. If so, please complete section C 5.1-5.1.5 in the revised protocol.

   - The animals will be not anesthetized for the IV injection of tumor cells.
   -

g. Please indicate when in relation to tumor development will the treatment begin.

   - Treatment will begin tumor grow to 40 mm^3 or two week of iv transplantation.
   -

h. The protocol application indicates in section C 1.0 that "We also propose to use TUBO cells, a mouse carcinoma cell line expressing human HER2 (29) to establish a congenic HER2+ mouse carcinoma model to further characterize therapeutic function of cytokine-associated TRA/GO in a immunocompetent host." However, this does not appear to be described in the application. Please clarify and/or provide a complete description of this part of the study.

   - Experiments using TUBO is proposed as future directions, and will not be performed under the current protocol.

17. Section C, 5.2.2, Indicate the endpoints(s) in time when animals are scheduled to be euthanized for each study group (i.e., hours/days/ or weeks).
    a. The box to indicate that no experiments are performed on live animals, only euthanasia and tissue harvest was checked. Please clarify.

       - Live animals will receive treatment and blood collect as described.

18. Section C, 6.2, Describe any of the expected symptoms that might become painful (i.e., toxicity, tumor size, disease progression) and indicate when animals will be euthanized for humane reasons.
    a. Please further describe the tumor progression (lymphoma) and its effects on the animal's health and well-being.

       - At early stages, the animal's health and well-being will not be affected by the lymphoma. At late stage, lymphoma will cause paralysis or weight loss. When this occurs or weight loss reaches 20% of the body weight, the animal will be euthanized.
       -

    b. If applicable, please describe the potential effects of irradiation on the animal's health and well-being and indicate end points that may be used to early remove an animal from study.

THE UNIVERSITY OF UTAH

- The mice will not be irradiated. Correction is made.
-

19. Section C, 7.2, List all other personnel working on the study.
    a. The protocol application indicates that the listed personnel will be responsible for anesthesia (A) and surgery (S). However, anesthesia and surgery do not appear to be a part of this study. Please clarify and/or correct this section in the revised protocol.

        - Corrected

20. Attending veterinarian has helped plan the activities of this protocol. Our standard operating procedure for protocol development requires that the protocol be developed with the assistance of the U of U veterinarian.
    a. The protocol application has been assigned to [redacted] The protocol application and the protocol prescreen will be sent to him. Please be sure to work directly with the veterinarian to complete this requirement.

        - The revised protocol is send to [redacted] for review.
        -

    b. Please provide assurance that the revised protocol, with changes listed above, has been reviewed and approved by [redacted]

4/14/17 [redacted]

Claims Detail Report
Date Run: June 20, 2017

Policy Information

Name: UNIVERSITY OF UTAH
Policy Number: 1635568
Location Name: UNIVERSITY OF UTAH
Department Nbr: [redacted] SOM

Status Information

Claimant Name: [redacted]
Claim Number: 201707760
Date of Injury: 04/17/2017
Date Reported: 04/25/2017
Date Created: 04/26/2017
Adjuster Name: [redacted]
Claim Status: Open Med **PHONE:** [redacted]
RTW Date:
Class Code: 9101 -COLLEGE OR SCHOOL ALL OTHER EMPLOYEES
Litigation: **Subrogation:** N

Injury Description

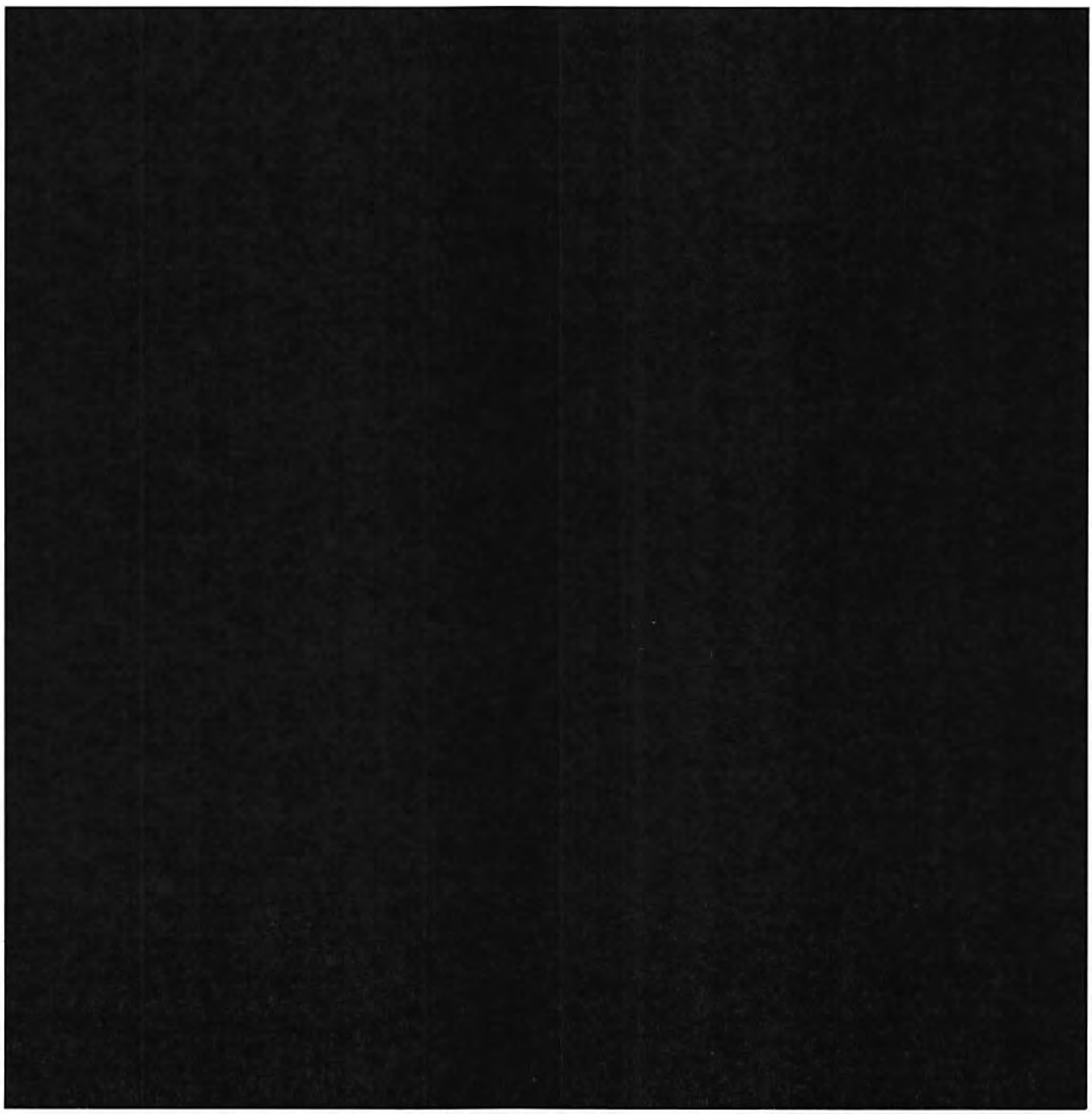
Part of the Body: Hand
Nature of Injury: Puncture
Accident Desc: Animal Or Insect
Accident Description: A mouse bit the employee after an hPBMC injection.
No Permanent Indemity paid on the claim

Claim Costs

	PAID	RESERVED	RECOVERED	INCURRED
COMP:	.00	.00	.00	.00
TTD:	.00		.00	
TPD:	.00		.00	
PPD:	.00		.00	
PTD:	.00		.00	
OJT:	.00		.00	
REH:	.00		.00	
FAT:	.00		.00	
MED	.00	459.00	.00	459.00
REHAB	.00	.00	.00	.00
ALAE	.00	3.00	.00	3.00
			TOTAL INCURRED	462.00

U of U Institutional Animal Care and Use Committee (IACUC) Meeting Minutes
April 26, 2017

IACUC MEMBERS PRESENT		NOT PRESENT
#1	#3	#7
#9	#11	
#17	#18	
#19	#20	
#22	#24	
#25	#26	
#27	#28	



[redacted]

13. Protocol Review

As each protocol was individually reviewed and discussed by the committee, the IACUC chairman initially provided a brief verbal summary of the protocol. The summary included the title of the protocol, the number of animals, the species, and the pain categories they were assigned. All of this information is found in the written protocol application and not restated here. Any additional committee discussions and deliberations are listed below by protocol. All committee member deliberation votes were unanimous unless noted below.

THE FOLLOWING PROTOCOLS WERE APPROVED

*Convened IACUC meeting vote with a quorum present.

[redacted]

THE FOLLOWING PROTOCOLS WERE APPROVED TO DESIGNATED MEMBER REVIEW

*The DMR reviewer is the IACUC chair (or designee) and IACUC director (Both are IACUC members).

[redacted]

[redacted]

Committee member # 20 left the meeting and did not return.

[redacted]

[redacted]

17-04018 A Novel Antibody Formulation for Cancer Treatment

Questions

1. Please provide the IBC approval letter for the use of human tumor cells in this research project.
2. Please provide the OEH&S approval for the use of chemotherapeutics in this research project.
3. The committee determined that the 129 study animals should be placed in category C (non-painful procedure). Please indicate if this is acceptable.

THE FOLLOWING AMENDMENTS WERE APPROVED TO DESIGNATED MEMBER REVIEW

*The DMR reviewer is the IACUC chair (or designee) and IACUC director (Both are IACUC members).

[redacted]

THE FOLLOWING PROTOCOLS WILL COME BACK TO THE COMMITTEE

[redacted]

THE FOLLOWING AMENDMENTS WILL COME BACK TO THE COMMITTEE

[redacted]

[redacted]

IACUC MEMBER NOTIFICATION OF CONTINUING REVIEW OF PROTOCOLS (Conducted no less than annually)

[redacted]