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Reading: IS00005974

Study Identification

1.1

The title and narrative of this IACUC application must match the title and narrative of the grant that supports this research.

Submit a separate IACUC application for each federal or major agency grant.

1.1.1 * **Main Title** >>Get Help
In Vivo Models of Leptomeningeal Metastasis using Established Cell Lines and Patient Derived Xenografts (PDXs) of Cerebrospinal Fluid (CSF) Circulating Tumor Cells (CTCs)

1.1.2 **Additional Titles:** >>Get Help
Novel Immunotherapy for Brain-Metastatic Breast Cancer
Defining and targeting leptomeningeal melanoma metastases (MPI)

1.1.3 * **Principal Investigator:**
The applicant Principal or Secondary Investigator must have a faculty appointment with the University or an appropriate appointment with the James A. Haley Veteran's Administration Medical Center, H. Lee Moffitt Cancer Center and Research Institute, or New College of Florida.
Peter Forsyth

1.1.4 **Secondary Investigator(s):**

First Name	Last Name	Organization	IACUC Cert Expiration	Profile
Keiran	Smalley	H Lee Moffitt Cancer Center	11/1/2021	00001582
Inna	Smalley	H Lee Moffitt Cancer Center	8/9/2027	00007092

1.1.5 **Research Staff:**
List all IACUC-certified personnel that will contribute to the study. >>Get Help

Last Name	First Name	Organization	IACUC Cert Expiration	Profile
Aceti	Massimiliano	H Lee Moffitt Cancer Center	10/12/2024	00021056
Kodumudi	Krithika	H Lee Moffitt Cancer Center	9/15/2027	00006638
Law	Vincent	H Lee Moffitt Cancer Center	1/27/2023	00016893
Ramamoorthi	Ganesan	H Lee Moffitt Cancer Center	2/5/2024	00019539
Ramello	Maria	H Lee Moffitt Cancer Center	4/12/2023	00017268
Snyder	Colin	H Lee Moffitt Cancer Center	11/16/2023	00018943

1.1.6 * **List Secondary Study Contact(s):**
Secondary study contacts can manage the correspondence of a protocol in the absence of the PI. >>Get Help

Person	Organization
Vincent Law	H Lee Moffitt Cancer Center

1.1.7 * **Please Select:**
○ New Project
● **3rd year renewal replacing a previously approved protocol**

Protocol Continuations

1.2

1.2.1 * **Please enter the number of the previous protocol this application is replacing.**
IS00001909

1.2.1a **Upload the previous protocol:**

Name	Modified	Version
Print_ IS00001909 - In Vivo Models of Leptomeningeal Metastasis using Established Cell Lines and Pat.pdf(0.01) ···	12/3/2018 9:11 AM	0.01

1.2.2 * **For research applications ONLY, summarize work accomplished during the prior three-year approval period and how this proposal extends those findings. (Indicate Not Applicable, N/A, for Murine Colony, Antisera Production or Tissue applications).**
We successfully generated a brain metastasis/ LMDz model by injecting GFP/Luc-tagged murine- and human-derived melanoma cell line (WM164 and SM1) into the cisterna magna of immunocompromised (NSGs and Nude) mice. We performed a drug screen trial on these tumor bearing mice. Further investigation is needed to validate our preliminary finding.

On the other hand, tumor development was absent in immunocompetent (BL/6) mice, possibly due to cells labelled with GFP/Luc.

In addition, we have yet been able to generate brain tumors in both immunocompromised and immunocompetent mice using ex-vivo melanoma cells extracted from patients. We are still working on optimizing the PDX model condition.

Study Personnel Certification Information

1.3

The following individuals' certification information must be current before IACUC approval can occur.
Please Note: The information on this form is read-only. An individual's certification information can be updated on his or her Researcher Profile. Verification dates are granted by the IACUC following review of submitted information

Principal Investigator:

Name	CV	Animal Experience		Documentation of Online Training Completion	Date Verified	Link to Profile
Peter Forsyth	Peter Forsyth CV(0.04) ···	Mouse	21	AALAS - USF Animal Reseach Orientation(0.02) ···	1/22/2016	00004562

Secondary Investigators:

Name	CV	Animal Experience		Documentation of Online Training Completion	Date Verified	Link to Profile
Keiran Smalley	CV for Dr. Keiran Smalley (0.01)	Mouse	15	KSmalley certificate.pdf(0.02)	11/1/2015	00001582
Inna Smalley	Inna Smalley CV(0.03)	Mouse	5	Inna Fedorenko Online Training Certificate(0.03)	8/9/2021	00007092

Research Staff:

Name	CV	Animal Experience		Documentation of Online Training Completion	Date Verified	Link to Profile
Massimiliano Aceti	Curriculum Vitae(0.01)	Mouse	17	AALAS Laws-Regulations-Policies-Guide - USF Orientation - Certificate.pdf(0.01)	10/12/2018	00021056
Krithika Kodumudi	Krithika Kodumudi CV 2017.doc(0.02)	Mouse	15 yrs	AALAS Learning Library - Exam Certificate.pdf(0.02)	9/15/2021	00006638
Vincent Law	Vincent Law Resume(0.02)	Mouse	7	AALAS Learning Library Certificate of Training.pdf(0.01)	1/27/2017	00016893
Ganesan Ramamoorthi	CV-Ganesan.pdf(0.02)	Rat Mouse	4 8	CEUCertificate.pdf(0.01)	2/5/2018	00019539
Maria Ramello	RamelloMC Biosketch 2017(0.01)	Mouse	8	COI certificate of completion - RamelloMC(0.03)	4/12/2017	00017268
Colin Snyder	Resume(0.01)	Mouse	4	AALAS Animal Handling Certificate(0.03)	11/16/2017	00018943

Funding Information

2.1

2.1.1 * **Select the funding source which best describes the sponsor of this research (check all that apply):** >>Get Help
Federal government or major agency that awards grants based on peer-reviewed proposals (NIH, NSF, DOD, AHA, ACS, etc.)
Non-Profit (Private Foundations, H. Lee Moffitt Cancer Center, etc.), For Profit (Industry Sponsored) or Other
USF department, institute, center, etc.

2.1.2 * **Does the funding source/sponsor of this study require compliance with 21 CFR 58 Good Laboratory Practices (GLP) Standards?** >>Get Help
○ Yes ● **No**

2.1.3 * **Will this protocol use any VA resources?**
○ Yes ● **No**

2.1.4 * **Will this protocol be supported by funds administered by Moffitt Cancer Center?**
● **Yes** ○ No

Funding - Federal/Major Agency

2.2

2.2.1 **Indicate the federal agencies that award grants based on peer-reviewed proposals:** >>Get Help
National Cancer Institute

2.2.1a **Other federal or major agency:**
DOD

2.2.2 * **Please enter the grant or contract number:**
Pending

2.2.3 * **Upload the federal or major agency grant narrative, or portions that include the face page and research plan which describes the specific aims and methods involving vertebrate animal use.** >>Get Help

Name	Modified	Version
Face%20Page.pdf(0.01) ···	2/12/2019 10:14 AM	0.01
Grant%20Proposal.pdf(0.01) ···	2/12/2019 10:14 AM	0.01
Smalley-Forsyth%20R21%20FINAL%20SUBMITTED.pdf(0.01) ···	12/3/2018 9:57 AM	0.01
vertebrate%20animals%20final%20for%20R21.docx(0.01) ···	12/3/2018 9:58 AM	0.01

Funding - Non-Profit, For Profit, or Other

2.3

2.3.1 **Indicate the Non-Profit, For Profit or Other funding support source:**

2.3.1a **Other Industry:**
Foundation Funds: "Golfers vs. Brain Cancer" 09-33603-18-01

Protocol Type

3.1

3.1.1 * **Select the option that best describes your protocol type:** >>Get Help

Type
● **Research or Teaching**
○ Wildlife Research
○ Murine Colony Only
○ Antisera Production (Rabbit Only)
○ Tissue Use Only

Alternatives and Literature Search

4.1

4.1.1 * **On this page indicate that a search for alternatives and alternative methods has been conducted, including a search of at least two relevant databases (e.g., PubMed), covering the dates searched (i.e. at least the last ten years), using the indicated search term(s), and that the search was conducted on the indicated date. Two entries must be made to section 4.1.1, then respond to the following three sections.** >>Get Help

	Name of Database	Dates Covered	Key Words and Search Strategy	Date Search Conducted
View	PubMed	2008-2018	Mice, Rats, Research, Surgery, Analgesia, Anesthesia, Tumor, Melanoma, Melanoma Leptomeningeal Metastases, Melanoma Brain Metastasis, Alternatives, Surgery, Stereotactic, Intracranial, cranial vault cannula, imaging, pain, distress, early endpoint, euthanasia, Intracardiac injection, Intrathecal Injection.	12/03/2018
View	BioOne	2008-2018	Mice, Rats, Research, Surgery, Analgesia, Anesthesia, Tumor, Melanoma, Melanoma Leptomeningeal Metastases, Melanoma Brain Metastasis, Alternatives, Surgery, Stereotactic, Intracranial, cranial vault cannula, imaging, pain, distress, early endpoint, euthanasia, Intracardiac injection, Intrathecal Injection.	12/03/2018

4.1.2 * **Indicate in a narrative below that in the search that non-animal alternatives were considered, that less sentient species were considered, and that none were found suitable. (The narrative must demonstrate that alternatives to the use of animals as described in this application are not available.)** >>Get Help
Our literature search for possible non-animal alternatives was examined and additionally less sentient species were considered, but were not suitable because we are conducting pre-clinical research examining metastatic brain cancer treatments using experimental therapeutics.

4.1.3 * **Indicate in a narrative below that alternatives to painful procedures were considered, none were found suitable, and that methods described will be continuously refined so as to reduce animal discomfort. (The narrative must demonstrate that alternatives to these procedures as described in this application which may cause pain or discomfort to animals are not available or suitable.)** >>Get Help
We searched the literature in consideration of alternatives to painful procedures. None were more suitable or less painful/less distressful alternatives that could accomplish the goals of our animal use protocol. We will continuously refine our procedures so as to reduce animal discomfort.

4.1.4 * **Indicate in a narrative below that this animal use does not unnecessarily duplicate previous research or existing studies:** >>Get Help
When searching Pubmed or BioOne for "CSF Circulating tumor cells in leptomeningeal disease in vivo", no items are found. This research study is based on new in vitro results from our laboratory.

1

Characteristics of Animals and Pain Category of Research

5.1

List and describe the animals to be used. Indicate the anticipated number of animals to be used in each Pain Category of Research and the total number of animals involved during the 3-year approval period of this protocol. Indicate strain or line designation if rodents are requested. Indicate the number of each species/strains that will be involved in planned procedures that are anticipated to produce momentary, slight, or no pain, discomfort or distress (USF Pain Category A = USDA Pain Category C); more than momentary or slight pain, discomfort or distress which is alleviated by the use of appropriate anesthetics and/or analgesics (USF Pain Category B = USDA Pain Category D); or pain, discomfort, or distress, which cannot, or is not alleviated by the administration of appropriate anesthetics and/or analgesics (USF Pain Category C = USDA Pain Category E). (Note: Refer to IACUC Principles & Procedures XII.)

5.1.1 * **For each animal group, click +Add and complete the form.**

	Species / Stock/Strain / Characteristics	# Pain Category C	# Pain Category D	# Pain Category E	Total Number
View	Mouse: BL/6 (6-10 weeks/Male and Female)	0	0	1040	1040
View	Mouse: NSG (6-10 weeks/Male and Female)	0	0	186	186
View	Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female)	0	0	186	186
View	Mouse: BALB/cJ (6-10 weeks/Male and Female)	0	0	1880	1880
View	Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	0	0	180	180

If any additional strains have been added at Amendment, they will be shown below:

Housing and Use Sites

6.1

6.1.1 * **Will animals be housed in a University-managed animal facility?**
● **Yes** ○ No

6.1.2 * **Will animals be transferred from the housing room to any animal research/procedural/testing areas outside of the University-managed animal facility?**
○ Yes ● **No**

6.1.3 * **Does this proposal involve an inter-institutional collaboration or will be supported by a subcontract from another institution, or will involve animals housed at, or on behalf of another institution?**
○ Yes ● **No**

Housing Facilities

6.2

6.2.1 * **For each HOUSING FACILITY where animals will be housed, click +Add and complete the form.** >>Get Help

	Location	Species
View	SRB – Stabile Research Building	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)

2

Study Summary and Animal Use Rationale

7.1

7.1.1 * **Very briefly summarize (less than 150 words) in lay terms that are intelligible to a nonscientist (1) the purpose of the study, (2) the sequential procedures involved (*i.e., in general terms*), and (3) why the study is important to human or animal health, the advancement of knowledge, or the good of society:** >>Get Help
The purpose of the study is to examine the cause of leptomeningeal metastases of melanoma Leptomeningeal metastases of melanoma are a very dangerous condition and these patients develop severe neurological problems and will die within 8-10 weeks. In order to develop therapies, it is of very important for scientists and physicians to understand the factors responsible for melanoma tumor development and its leptomeningeal metastases. Here, we develop animal model study leptomeningeal metastases by using melanoma cells and circulating tumor cells (CTCs) collected from cerebrospinal fluid of leptomeningeal patients. Briefly, we will carry out stereotaxic surgery to intrathecally inoculate cancer cells into the brain of mice. The tumor will grow to after inoculation then animals will be given chemo drugs or immunotherapy treatments, monitored daily, and euthanized once they reach study end points. This proposal will help to understand the disease and also to develop new therapies against this disease.

Research for DOD:
Cancer vaccine treatments:
The induction of strong of anti-tumor immune responses can lead to rejection of tumors. The most important immune cell for tumor rejection is T cells. For the activation and expansion of tumor-specific T cells, dendritic cell (DC) vaccination approach has been used previously by our group and leads to strong immune responses against tumors and tumor rejection. In these studies, DC will be used to treat mice bearing brain metastasis and LMDz. We will also examine the anti-tumor efficacy of DC vaccines for complete tumor regression. Regression of tumors and survival will be measured. In addition, activation of immune cells (T cells) will be examined. Understanding the mechanism of DC vaccine and the combinatorial approach may increase the potential to be used in patients with cancer

7.1.2 * **Provide a narrative that indicates the design of this study considered the impact of the proposed procedures on the animals' well-being and balance that with the specific aims of research. (Explain how the scientific benefits of the intended research outweighs harm to the animals):**
Mice are necessary for testing to determine if CSF Circulating tumor cells are important in melanoma Leptomeningeal metastasis and if they might be a clinical-relevant. Thus, this proposal will provide results that will greatly benefit human health. A literature search has been performed to ensure that this procedure is not a replication of a well documented procedure and that alternatives to pain and distress and the 3 R's have been taken into consideration.

7.1.3 * **Describe the characteristics of each species/strain requested that justify its use in this protocol. If a specific sex is required, explain why:**
NSG, nu/nu (Nude) mice: Immunocompromised and possess an ability to accept and grow xenografts without rejection, hence permitting the growth of human CSF-CTCs in these animals.

BALB/c and BL/6 mice: Immunocompetent mice use for experimental controls and good candidates for immunology studies

Experimental Design

8.1

8.1.1 * **Provide a clear and concise description of the specific experimental manipulations and treatments of animals in chronological order so that IACUC reviewers understand exactly what will be done to all animals from entry into the study to its endpoints:** >>Get Help
Leptomeningeal disease (LMDz) is a very poorly understood disease and what factors mediate LMDz are not known. Therefore, the goals of this study are to: 1) develop animal models of LMDz using established melanoma cell lines. 2) determine if it is feasible to grow human CSF-CTCs derived from patients in murine and racine models. 3) Determine the basic characteristics of these models such as proportion of successful innoculataions, survival, imaging characteristics (IVIS200), morphological and molecular characteristics of CSF-CTCs that grow successfully (e.g. melanoma markers, Braf status, single cell analysis etc.).

We have IRB approval to conduct these studies under MCC 50103 "Ex vivo Characterization and Culture of Cerebrospinal Fluid–Circulating Tumor Cells (CSF-CTCs): A Feasibility Study To Optimize Collection, Analysis and Culture of CSF-CTCs Patients with Leptomeningeal Disease (LMDz) from Melanoma or Melanoma Brain Metastases (MBMs)."
We plan to use following three types of cells in this protocol to study melanoma LMDz.
1. We will use fresh CTCs isolated from CSF of melanoma LMDz patients as described in MCC 50103 and inject them into animals the same day as they are collected: We will collect CSF from the patients, spin down the CSF at 1500 rpm for 5 min and will suspend the cellular pellet in USP grade PBS, count the cells and bring up the final cell number approximately 10, 000 cells/microliter, and suspend them in media with growth factors before injecting them into the CSF space, via the cisterna magnum, into animals.
2. We will use ex vivo cultured CSF-CTCs from melanoma LMDz patients: We have already cultured CTCs from CSF in five patients. In brief, CSF collected will be spun down at 1500 rpm for 5 min, cellular pellets will be suspended in stem cell culture media and longer term cultures will be grown in human stem cell media supplemented with growth factors such as EGF, FGF and NGF. As we are optimizing culture conditions these will be grown in normoxic and hypoxic (~4% O2). Then cells will be engineered to express either a fusion of GFP-luciferase or a fusion of mCherry-luciferase and grown under the same conditions.
3. We will also use two established melanoma cell lines. These established melanoma cell lines will be cultured in RPMI media with serum and will be engineered to express either a fusion of GFP-luciferase or a fusion of mCherry-luciferase. We will also use unlabeled melanoma cell lines for the immune-competent models.

Please note that GFP-Luciferase or mCherry-Luciferase expressing constructs will be introduced into cells via standard transfection techniques. GFP-Lucifarse or mCherry-Luciferase constructs are not given to mice. The purpose of labeling these cells is to trace/visualize the growth of tumors by different readouts, i.e. GFP-Fluorescence and Luciferase-based chemiluminescence or mCherry based fluorescence and luciferase-based chemiluminescence.

We will use following animal models in this study and will carry out stereotaxic surgery to intrathecally inject the above mentioned cells to study LMDz:
1. NSG mice
2. Nude mice
3. BALB/c mice
4. BL/6 mice

Procedure: Six to ten week, mice will be injected with up to 50,000 cells diluted in 5 microlitre of USP grade PBS intrathecally. Cells will first be harvested by trypsinization and then washed three times in cold UPS grade PBS and then injected in a total volume of 5 microlitre by using a 30g needle w/silicone bead. Anesthetized rodent is placed into a small animal stereotaxic with the nose pointed down and positioned to get an approximate 1100–1300"bend" to open up the gap between the skull and C2. Insertion of a small gauge needle (30g mice) into the cisterna magna is performed (the triangular space between the back of the cerebellum and the pyramids, just anterior to the area postrema) and the needle advanced just until cerebral spinal fluid (CSF) is seen within the attached silastic tubing. Tubing is attached in turn to a syringe and 5ul of CSF aspirated, a new syringe containing a replacement volume of fluid (e.g.: Evan's blue for assessment) is attached and delivered. Two approaches will be modeled; either a percutaneous stick through the skin, or to better visualize blood vessels, via surgical approach and minimal blunt dissection of the muscles just over the cisterna magna. An incision of ~1cm will be made beginning mid-skull and extending dorsally; the muscles will be gently dissected and retracted enough to visualize the dura mater and create a clear window for placement of the needle. The surgical incision will be closed with sterile stainless steel wound clips. Animal will be recovered to assess for obvious signs of injury (e.g.: ataxic, staggering, labored breathing, etc.) for up to 48hrs; animals may be euthanized to assess Evan's blue placement within the tissue.

Tumor-bearing animals will be scanned weekly for fluorescence or luminescence as needed. Prior to imaging animals will be injected (IP) with 10 ul per g body weight of sterile d-luciferin substrate prepared in PBS at 15 mg/ml (resulting dose 150 ug/ g body weight). Then hair on the animals will be removed with the topical application of Nair, Nair will be applied for < 30 seconds, wiped off and skin cleaned with alcohol. Tumor growth will be characterized by weekly fluorescence or luminescence measuring using the IVIS 200 in vivo fluorescence/luminescence imaging system. CSF-CTC cells from patients, inoculated into the CSF on the same day, will be unlabeled by necessity and followed until symptoms/signs or a loss of more than 20% body weight occurs. Once there is imaging or behavioral/weight loss, evidence of significant tumor growth the animals will be anesthetized and sacrificed. If necessary, because of no obvious tumor growth, CSF will be collected at autopsy and injected back into mice, respectively. CSF from all animals will be re-inoculated or recycled into new animals 3-5 times.

To sustain a small number of CSF-CTCs PDXs, we will inoculate the minimum number of CSF-CTCs to maintain these CSF-CTC PDXs for up to a year in a maximum of three mice per patient. We will estimate the minimal number of cells needed (depending on the above experiments) to maintain the CSF-PDX and prolong survival to minimize the number of animals we use. For example, one CSF-CTC from one patient may be particularly aggressive when 50,000 cells are inoculated and so we will attempt inoculation with 50,000 cells then 10,000 and 1,000 cells to determine the minimal number that produce a successful inoculation. For example, animals with 50,000 cells may survive for one month but those inoculated with 1,000 cells may survive four months. Determining this would allow the CSF-CTC PDX to be perpetuated but will also minimize the number of animals used.

For immune-competent models of LMDz we will use melanoma cell lines which are not labeled. We would therefore track tumor growth based on previously observed tumor growth dynamics for this cell line coupled with observation for neurological symptoms and behavioral changes in the mice. We have previously observed that the untreated SM1 model of leptomeningeal metastasis has a fairly consistent and predictable rate of progression over 4 weeks in mice. We may also confirm tumor growth using MRI/CT imaging. Due to rapid progression of this model, we would begin drug treatments in animals immediately after tumor cell implantation.

For drug treatment study:

i) Drugs will be administered when signs of tumor growth is evident, characterized by positive luminescence signal (usually at 1.5 to 2 weeks). In cases of unlabeled tumor cell inoculations, treatments will begin within first week post inoculation. Drugs are given either per os by garage, or by mixing in the feed, or by intraperitoneal injection, depending on the compound. Based on our in vitro data associating specific genetic mutations with specific drug combination efficacies, we will design each drug trial so as to translate in-vitro findings to in-vivo relevance.

The chemo compounds for our experiment include: Dabrafenib is a novel inhibitor of BRAF V600E. Trametinib is a novel inhibitor of MEK. Ceritinib is a novel inhibitor of ALK. Galunisertib (LY2157299) is a TGF-beta specific inhibitor. These compounds will be administered by oral gavage or through drug-formulated chow daily. Liposomal Chlodronate suspension is used for depletion of macrophage cells and will be administered by IP. Immune checkpoint inhibitors include anti-PD1 antibody.

Vehicles/controls will include (Water 99% + 2 Hydroxyethyl cellulose 0.5% + Hydrochloric Acid 5mM + Polysorbate-80 60ppm), or sterile saline, or (10% DMSO + 1% Carboxyethylcellulose salt/water solution) or (CremophorEL 12.5% + Ethanol 12.5% + Water 75%) or MCT (0.5% methylcellulose, 0.2% Tween-80) or corn oil, or 0.5% HPMC + 0.2% Tween-80 in water, or 0.5% dextrose in water depending on the drug or compound administered, as recommended by the manufacturer. We will also use liposomal suspension, without chlodronate for the liposomal chlodronate treatments and antibody isotype controls for immune checkpoint inhibitor treatments.

In all cases, USP grade drugs, vehicles, novel compounds and chemotherapeutics will be used whenever available.

ii) Evaluation of CAR-T cell therapy in a LMDz model of melanoma brain metastasis. Animals will be injected with the melanoma cell line (A375ffLuc) into the CSF space (intrathecal injection). Each experimental cohort will be n=10 mice. A375 melanoma cells @ 5x10e5 cells in 5ul will be injected into the CSF space via the cisterna magnum. 4 days after tumor cell injection, some mice will undergo stereotaxic surgery to implant a cannula (a.k.a Top Hat) for intraventricular delivery of CAR-T or control T cells treatment [@ 1x10e5 in 2ul. Other group of mice will receive CAR-T or control T cells treatment i.v. 7 days after tumor cell injection [via the tail vein @ 10x10e6 in 100ul].
For this experimental condition n=10 mice x 1 cell line x 2 T cell products (CAR-T or mock-T control) + 1 vehicle administered control cohorts x 2 routes of treatment delivery x 3 times repeated = 10 x 1 x 3 x 2 x 3= 180 mice required.

Research below funded by the DOD

i) Vaccine Preparation: Dendritic cells will be harvested from euthanized C57BL/6 donor mice by isolating hematopoeitic stem cell progenitors from the bone marrow of long bones. To obtain an enriched DC isolate, these cells will be cultured in vitro in the presence of granulocyte/monocyte colony stimulating factors (GM-CSF) and interleukin-4 (IL-4) prior to their use.

ii) Total mice for this project are: 1880 Balb/C mice and 880 C57BL/6 mice

1. To generate HER2/HER3 DC1 vaccines from mice, we will require 120 C57BL/6 mice and 100 Balb/C mice per year

2. To evaluate the efficacy and mechanism of HER2 DC1 vaccines, total 120 Balb/C mice will be required

1a) To optimize the dose titration of HER2-DC1 vaccines subcutaneously versus intrathecally (N=10 mice per group) - Total 40 Balb/C mice will be required.
a. Control
b. HER2 peptide pulsed DC1 vaccine (100,000 cells)
c. HER2 peptide pulsed DC1 vaccine (50,000 cells)
d. HER2 peptide pulsed DC1 vaccine (25,000 cells)

1b) To evaluate and compare the efficacy of HER2- DC1 vaccines on TUBO cells via subcutaneous vs. Intrathecal (IT) models (N=10 mice per group) - Total 40 BALB/c mice will be required
a. Control
b. HER2/DC1 vaccine Sub-Q
c. HER2/DC1 vaccine IT
d. HER2/DC1 vaccine Sub-Q + IT

80 mice X four independent experiments= 320 Balb/C mice

3. To evaluate the efficacy and mechanism of HER3 peptide DC1 vaccines in TNBC model E0771 in C57BL/6 mice and 4T1 in Balb/ C mice- (N=15 mice per group X 4 groups)- Total of 120 C57BL/ mice and 120 Balb/C mice will be required

3a) To optimize the dose titration of HER3 peptide DC1 vaccines subcutaneously versus intrathecally (N=15 mice per group) - Total 60 Balb/C mice and 60 C57BL/6 mice will be required.
a. Control
b. HER3 peptide DC1 vaccine (100,000 cells)
c. HER3 peptide DC1 vaccine (50,000 cells)
d. HER3 peptide DC1 vaccine (25,000 cells)

3b) To evaluate the efficacy of HER3 DC1 vaccines in TNBC model E0771 in C57BL/6 mice and 4T1 in Balb/ C mice- (N=15 mice per group) - Total 60 Balb/C mice and 60 C57BL/6 mice will be required.

a. Control
b. HER3 peptide DC1 vaccine Sub-Q
c. HER3 peptide DC1 vaccine IT
d. HER3 peptide DC1 vaccine Sub-Q + IT

120 Balb/C mice X four independent experiments= 480 Balb/C mice
120 C57BL/6 mice X four independent experiments= 480 C57BL/mice

4. To examine the efficacy and mechanism of DC1 vaccines in combination with CKM and checkpoint inhibitors, (N=15 mice per group)- Total 10 groups X 15 mice= 150 Balb/C

a. Isotype/ Controls
b. CKM adjuvant
c. anti-PD-1
d. anti-PD-L1
e. HER2 peptide DC1 vaccine
f. HER2 peptide DC1 vaccine + CKM
g. HER2 peptide DC1 vaccine + anti-PD-1
h. HER2 peptide DC1 vaccine + anti-PD-L1
i. HER2 peptide DCI vaccine + CKM + anti-PD-1
j. HER2 peptide DCI vaccine + CKM + anti-PD-L1

150 Balb/C mice X four independent experiments= 600 Balb/C mice

iii) The experimental design and timeline for vaccine treatments are as follows:
Day 0: Injection of tumor cells in the cisterna magna
Day 4: Implantation of the ICV injectable port
Day 7: Intraperitoneal (IP) injection of monoclonal antibodies once a week for four weeks
Day 9: Sub-cutaneous (Sub-Q) or IT or both DC vaccination, follow-up with or without CKM or PD-L1 antibody or both once a week for three weeks

iv) DC vaccination:
For HER2+ with TUBO cells (BALB/c): DC vaccine pulsed with HER2 peptides.
For TNBC with either E0771 cells (BL/6 mice) or MT901 cells (BALB/c): DC vaccine pulsed with HER3 or CMET peptides.

***If the following mice that were given DC vaccination and/or HER2+ show improvement from disease progression (ie: undetectable tumor mass in the CNS region) for 20 days or more, then mice may be subjected for cancer re-challenge via cisterna magna or sub-Q injections of TUBO, E0771 or MT901 cells. Specifically, the mice that require re-challenge would already have an initial surgical approach of cisterna magna injection of cancer cells, and showing undetectable tumor mass in the CNS region after 20 or more days post-treatment. The re-challenge procedure will also be a surgical approach of injection of the same cancer cells. Re-challenged mice will be monitor once a week via IVIS to determine tumor growth. We will only monitor tumor progression and not administer any treatments on re-challenged mice. End-point criteria will be the same as below.

During treatment trial, mice will be observed daily for signs of discomfort or distress, or weight gain or loss, for up to 6 weeks or until clinical end points are met. Mice that lose more than 20% or gain more than 10% of their original body weight (measured twice weekly), or show signs of discomfort or distress (e.g., hypoactivity, cyanosis, pale mucous membranes, head pressing, head tilt, circling, impaired locomotion, non-purposeful movements, failure to groom, failure to respond to stimuli, isolation from cage mates, shivering, ataxia, shallow, rapid and/or labored breathing, soiled anogenital area, vocalization, lack of inquisitiveness, and/or a hunched posture) will be euthanized. Tumor progression during treatment trial will be assessed using the IVIS imaging system, once or twice per week.

8.1.2 **For complicated experimental designs, upload a detailed flowchart, diagram, or table, which depicts the experiments or sequence of events with a timeline, and indicates the number of animals needed for each experimental group and the number of groups required:** >>Get Help

Name	Modified	Version
Schematic%20for%20IACUC%20112515-RK-grouped.pptx(0.01) ···	12/3/2018 10:30 AM	0.01

Statistical Analysis and Rationale

9.1

The following species/strains/lines were requested in Section 5. Use this table to complete your response to the questions below: >>Get Help

Species / Stock/Strain / Characteristics	# Pain Category C	# Pain Category D	# Pain Category E	Total Number
Mouse: BL/6 (6-10 weeks/Male and Female)	0	0	1040	1040
Mouse: NSG (6-10 weeks/Male and Female)	0	0	186	186
Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female)	0	0	186	186
Mouse: BALB/cJ (6-10 weeks/Male and Female)	0	0	1880	1880
Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	0	0	180	180

9.1.1 * **Describe the statistical analyses and rationale used to determine experimental group sizes and the total number of each species of animals requested:** >>Get Help
Number of animals required as follows:

i) Using fresh CSF-CTCs from patients inoculated on the same day as they are collected: CTCs from a maximum of 6 patients will be used. This is a maximum number and we suspect the number of viable CSF-CTCs will be much smaller. For example, we have attempted to culture CSF-CTCs ex vivo in five patients and only 2 (40%) of these are growing. And we only collect a sample for one patient per month on average (this is a rare disease). So it remains likely that only five CSF-CTCs will grow in vivo of the 12 patients we anticipate collecting from. For each patient CSF-CTCs will use 3 for NSG mice and 3 for Nude mice, total of 6 animals/patient and total of 36 animals (18 NSG, 18 Nude). Re-injection of mice CSF-CTCs for 5 cycles requires 180 animals (90 NSG, 90 Nude). Total: 216 animals (108 NSG, 108 Nude).

Establishing and maintaining a CSF-CTC PDX from two patients: For CSF-CTC PDX will use two patients. For each patient's CTCs will use 3 NSG, and 3 Nude, total of 6 animals/CTC line and total of 12 animals (6 NSG, 6 Nude).

Using ex vivo- CTCs: CTCs cultured from 2 patients will be used. For each CTCs will use 3 NSG, and 3 Nude total of 6 animals/CTC line and total of 12 animals (6 NSG, 6 Nude). Reinjection of mice CTCs for 5 cycles requires 60 animals (30 NSG, 30 Nude). Total: 72 animals (36 NSG, 36 Nude).

Using established melanoma cell lines: There are several Human-derived melanoma cell lines WM1346, WM1366, WM1361A, M245, IPC-298, SKMel103, 1205Lu, M229, M249, WM793, 451Lu, WM164, WM983A, WM3918 and Mouse-derived cancer cell lines SM1. Based on the results of our ongoing in vitro experiments we will select two lines from the list. For each line we will inoculate 3 NSG, and 3 Nude for a total of 6 animals/ line and total of 12 animals (6 NSG, 6 Nude). Re-injection of mice CSF-CTCs for 5 cycles require 60 animals (30 NSG, 30 Nude). Total: 72 animals (36 NSG, 36 Nude).

Total =186 NSG + 186 Nude = 372 (NSGs and Nudes) animals.

ii) Evaluation of CAR-T cell therapy in a LMDz model of melanoma brain metastasis. Animals will be injected with the melanoma cell line (A375ffLuc) into the CSF space (intrathecal injection). Each experimental cohort will be n=10 mice. A375 melanoma cells @ 5x10e5 cells in 5ul will be injected into the CSF space via the cisterna magnum. 4 days after tumor cell injection, some mice will undergo stereotaxic surgery to implant a cannula (a.k.a Top Hat) for intraventricular delivery of CAR-T or control T cells treatment [@ 1x10e5 in 2ul. Other group of mice will receive CAR-T or control T cells treatment i.v. 7 days after tumor cell injection [via the tail vein @ 10x10e6 in 100ul].
For this experimental condition n=10 mice x 1 cell line x 2 T cell products (CAR-T or mock-T control) + 1 vehicle administered control cohorts x 2 routes of treatment delivery x 3 times repeated = 10 x 1 x 3 x 2 x 3= 180 mice required.

iii) Research for DOD:
We will perform the following experiments to address our research aims on LMDz. Certain aims will require more than 15 mice per group because mice CSF come in very small quantity and we will need to pool them post-mortem for future immunology studies.

Aim 1. To generate Her2/Her3 DC1 vaccines from mice, we will require 160 BL/6 mice and 120 BALB/cJ mice.

AIm 2. To evaluate the efficacy and mechanism of Her2-DC1 vaccines
2a) To optimize the dose titration of Her2-DC1 vaccines sub-Q versus IT (10 mice per group x 3 independent experiments = 120 BALB/cJ): i) control, ii) Her2/DC1 vaccine (100,000 cells), iii) Her2/DC1 vaccine (50,000 cells), iv) Her2/DC1 vaccine (25,000 cells).

2b) To evaluate and compare the efficacy of Her2-DC1 vaccines on TUBO cells via Sub-Q vs. IT models (20 mice per group x 4 independent experiments = 320 BALB/cJ mice): i) control, ii) Her2-DC1 vaccine Sub-Q, iii) Her2-DC1 vaccine IT, iv) Her2-DC1 vaccine Sub-Q + IT.

Aim 3. To evaluate the efficacy and mechanism of Her3-DC1 vaccines in TNBC model E0771 in BL/6 mice and 4T1 in BALB/cJ mice.
3a) To optimize the dose titration of Her3-DC1 vaccines Sub-Q vs. IT (15 mice per group x 4 independent experiments = 240 mice for BALB/cJ and 240 mice for BL6): i) control, ii) Her3-DC1 vaccine (100,000 cells), iii) Her3-DC1 vaccine (50,000 cells), iv) Her3-DC1 vaccine (25,000 cells).

3b) To evaluate the efficacy of Her3-DC1 vaccines in TNBC model E0771 in BL/6 mice and 4T1 in BABL/cJ mice (30 mice per group x 4 independent experiments = 480 mice for BALB/c and 480 mice for BL6): i) control, ii) Her3-DC1 vaccine Sub-Q, iii) Her3-DC1 vaccine IT, iv) Her3-DC1 vaccine Sub-Q + IT.

Aim 4. To examine the efficacy and mechanism of DC1 vaccines in combination with CKM and checkpoint inhibitors (15 mice per group x 10 groups x 4 independent experiments = 600 BALB/cJ mice). i) isotope/control, ii) CKM, iii) anti-PD-1, iv) anti-PD-L1, v) Her2-DC1 vaccine, vi) Her2-DC1 vaccine + CKM, vii) Her2-DC1 vaccine + anti-PD-1, viii) Her2-DC1 vaccine + anti-PD-L1, ix) Her2-DC1 vaccine + CKM + anti-PD-1, x) Her2-DC1 vaccine + CKM + anti-PD-L1.

Total BALB/cJ in all aims: 120 (aim 1) + 440 (aim 2) + 720 (aim 3) + 600 (aim 4) = 1880 mice
Total BL/6 mice in all aims: 160 (aim 1) + 720 (aim 3) = 880 mice

Endpoints: 1) The rates of successful inoculation (number of animals in which CSF-CTCs grow/number of animals inoculated). This will be determined on the basis of imaging using the IVIS 200 and on histological evidence of CSF-CTC tumor growth on autopsy. 2) Survival will be measured and determined. 3) Tumors successfully inoculated will be assessed histologically using standards markers to assess their derivation (i.e. melanoma derived v.s. an inflammatory reaction or other process etc.) and characteristics. 4) Tumor growth and distribution within the CSF and CNS will be determined using imaging and histological examination at autopsy.

The statistics used will be descriptive. This protocol is largely a feasibility study and, depending on the rate of successful inoculation (which could be as low as 0% for patient derived CSF-CTCs but is anticipated to be > 60% for established cell lines), therefore power calculations are not possible. Instead we will follow this protocol for a year and then re-evaluate the data to determine feasibility and perform sample size calculations.

9.1.2 * **Provide an explanation/rationale for assigning the specific number animals to the different pain categories of research declared above:**
Although the earliest clinical end point will be utilized, all animals are classified in category C as tumors will develop in the brain and discomfort may not always be reliably recognized early and treated effectively.

Experimental Endpoints

10.1

10.1.1 * **Is animal death (excluding death from euthanasia) an intentional endpoint in this protocol?** >>Get Help
○ Yes ● **No**

10.1.1a **If Yes, explain why an earlier endpoint is not possible:**

10.1.2 * **Will animals be involved in procedures that are anticipated to produce more than momentary or slight pain, discomfort, or distress which cannot, or will not be alleviated by the administration of appropriate anesthetics or analgesics?** >>Get Help
● **Yes** ○ No

10.1.2a **If Yes, describe the potential consequences of experimental manipulations, and the precise clinical criteria that will be used to ensure timely intervention:**
The rates of successful inoculation (number of animals in which CSF-CTCs grow/number of animals inoculated). This will be determined on the basis of imaging using the IVIS 200 and on histological evidence of CSF-CTC tumor growth on autopsy. Survival will be measured and determined. 3) Tumors successfully inoculated will be assessed histologically using standards markers to assess their derivation (i.e. melanoma derived v.s. an inflammatory reaction or other process etc.) and characteristics. 4) Tumor growth and distribution within the CSF and CNS will be determined using imaging and histological examination at autopsy.

These endpoints will be assessed as follows. All animals may develop LMDz. Mice will be observed daily for signs of disease or discomfort. Mice will be weighed twice weekly on Monday and Thursday. Mice that lose more than 20% of body weight (rapid weight loss over two to four days; or progressive weight loss over a few weeks), or show signs of morbidity or distress (e.g. hypoactivity, cyanosis, pale mucous membranes, head pressing, head tilt, circling, impaired locomotion, non-purposeful movements, failure to groom, failure to respond to stimuli, isolation from cage mates, shivering, ataxia, shallow, rapid and/or labored breathing, soiled anogenital area, vocalization, lack of inquisitiveness, and/or a hunched posture) will be euthanized. They will be examined grossly and histologically for the extent of tumor in the CNS and in extra-CNS sites if appropriate at autopsy.

10.1.2b **If Yes, describe the frequency of animal observation:**
All animals (including cancer re-challenged mice) will be observed every 12 hours for 48 hours after surgery (in conjunction with administration of analgesic agents) followed by once daily. In addition, mice will be weighed twice weekly on Monday and Thursday after the inoculation of circulating tumor cells.

10.1.2c **If Yes, describe the intervention (e.g., treatment) used upon reaching the clinical endpoint, or indicate that animals reaching clinical endpoints will be euthanized (but do not describe methods of euthanasia here, as these will be requested under item 11.1.1b):** >>Get Help
Animals that reach the clinical endpoint will be euthanized.

Research for DOD
Note: Cancer re-challenged mice will have the same endpoint criteria and will be euthanized if clinical endpoint is met.

Euthanasia

11.1

11.1.1 * **Are animals euthanized?**
● **Yes** ○ No

11.1.1a **If No, for each affected species, describe the final disposition of the animals:**

11.1.1b **If Yes, for each affected species, describe the method of euthanasia and means of ensuring death, indicating dose and route if a chemical agent or provide justification if a physical method:** >>Get Help
Euthanasia by inhalation of carbon dioxide from a compressed tank source is achieved by exposure to increasing concentrations of CO2 (e.g., a displacement rate from 30% to 70% of the chamber volume/min will be used) to avoid or minimize discomfort or distress, and will be followed by the assurance of the cessation of cardiovascular and respiratory movements by prolonged observation at room air for longer than 10 minutes, or by employing a secondary method of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy. The chamber will not be prefilled. The chamber will be slowly filled. The chamber will be cleaned and dried between groups of animals. Death will be verified prior to carcass disposal.

Procedure Classifications

12.1

Indicate the procedures you will employ in this study from the list below.

* **Select all that apply:**

Section 13: **Husbandry & Caging:**
☐ Special Husbandry (Non-standard) or Housing at Satellite Locations
☑ **Food and/or Water Manipulation**
☑ **Special Caging (Non-standard)**
☐ Exemption from Required Species-specific Enrichment
☐ Dog Exercise
☐ NHP Enrichment

Section 14: **Drugs Administered for Clinical Care:**
☑ **Anesthetic, Analgesic, or Veterinary Therapeutic Administration (include drugs used during surgery)**

Section 15: **Test Substances Administered for Experimentation:** >>Get Help
☐ Biological Toxins/Infectious Agent
☑ **Chemical Agent: Vehicle/Diluent/Solvent**
☑ **Pharmacological Agent**
☐ Radioactive Isotope
☐ Adjuvant/Antigen
☑ **Tumor Cell Lines**
☐ rDNA
☑ **Primary Human Explants-Tissue, Cells, Fluids**
☑ **Other Agents**

Section 16: **Specimen Collection:**
☐ Specimen Collection Ante mortem
☑ **Specimen Collection Post mortem (PI acknowledges that animals will be confirmed dead before tissues are collected.)**

Section 17: **Surgery:**
☑ **Surgery (check Drug Administration above to account for drugs used during surgery)**

Section 18: **Other Experimental Procedures:**
☐ Irradiation
☑ **Diagnostic Imaging & Microscopy**
☐ Use of Human Patient Procedural Areas
☐ Breeding (Not Colony Production)
☐ Prolonged Restraint
☐ Stress Paradigms
☐ Behavioral Testing
☑ **Other Experimental Procedure (Not listed above)**

## Food and/or Water Manipulation

13.2

13.2.1 * **Name each affected species and describe all non-standard food and/or water manipulations (e.g. special dietary formulations, fluid supplements, food or water deprivation, calorie restrictions):** >>Get Help
NSG, Nude, BALB/c, BL/6 mice.

We need to provide these animals with fluorescence free diet prior to measuring fluorescence. Animals will be provided with fluorescent free diet 5V75 (containing Ground corn, ground wheat, wheat middlings, corn gluten meal,cane molasses). This diet will be fed 3 days before imaging.

Some mice will be administered the novel and conventional chemotherapeautic compounds by oral gavage.

Some mice will be administered pharmacological agents via rodent diet formulation, including:
Dabrafenib: 150 mg/kg diet dose
Trametinib: 1.5 mg/kg diet dose
Galunisertib: 500 mg/kg diet dose
The control animals will be fed the same diet without the small molecule inhibitors.

13.2.2 * **Justify the implementation of this non-standard food and/or water manipulation:**
Giving florescence free diet is important to measuring tumor specific florescence.

Drugs must be administered to tumor bearing mice to test relevance of in-vitro data regarding specific melanoma drug resistance and vulnerabilities in an in-vivo model.

The drug-formulated rodent diet route of administration is a lot less stressful for the animals than other modes of drug administration.

13.2.3 * **Describe the methods used to monitor animal condition during the procedure and methods used to avoid and alleviate discomfort, if anticipated:**
Not anticipated any discomfort by this food.

Mice will be monitored daily by animal care and research staff, weighed twice weekly, and in cases of luciferase-tagged tumors, they will be assessed via IVIS 200 once or twice per week.

Special Caging (non-standard)

13.3

13.3.1 * **Name each affected species and describe all non-standard caging used including the length of time housed (e.g., metabolic caging, wire mesh rodent caging, environmental chambers, other configurations):** >>Get Help
NSG and Nude mice.

13.3.2 * **Justify the implementation of this non-standard caging:**
These animals are immunocompromised, therefore sterile caging is required.

13.3.3 * **Describe the methods used to monitor animal condition during the procedure and methods used to avoid and alleviate discomfort, if anticipated:**
Animals will be monitored regularly

Drug Administration

14.1

14.1.1 * **Indicate the type(s) of drugs you will administer to animals during the course of this study.**
Anesthetics, analgesics and/or tranquilizers

14.1.2 * **Please press +Add and complete the chart below for each drug used in this protocol. (Please note that you will be asked to provide further information for each drug on subsequent pages.)** >>Get Help

	Drug	Pharmaceutical Grade?	Non Pharmaceutical Explanation	DEA Controlled?
View	Isoflurane (Forane)	yes		no
View	Ketoprofen	yes		no
View	Buprenorphine Sustained Release	yes		no
View	Meloxicam	yes		no
View	Ketamine/Xylazine	yes		yes
View	Carprofen	yes		no
View	Lidocaine	yes		yes

Anesthetics, Analgesics, Tranquilizers and Paralytics

14.2

14.2.1 * **Please press +Add and complete the chart below for each drug used as an anesthetic, analgesic, tranquilizer, paralytic or reversal agent. (Do not include drugs used for therapeutic purposes):** >>Get Help

	Drug	Species	Dose	Route	Administration Frequency	Treatment Duration	Monitoring Efficacy
View	Lidocaine	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	5 mg/kg	Sub-Q	Once before surgery	N/A	Pain medicationl that blocks signals of nerve endings in he skin before making an incision for surgery.
View	Buprenorphine Sustained Release	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	0.5-1.5 mg/kg	SC	once every 72 hrs	less than one min admin time	Analgesic; general appearance and observation indicating animal is painful (guarding, vocalization, rapid respiration rate, etc.) or behaving normal (posture normal, eating/drinking/voiding normal).
View	Carprofen	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	10mg/kg	SQ	Every 12 hours	4 days	Carpofen will be at 12 hour (or less) intervals for preemptive analgesia and post-op analgesia when Ketaprofen is not available. All animals will be monitored at regular intervals during the postoperative period following carpofen administration. If mice exhibit signs of post-operative complications (i.e., swelling, redness, guarding of the affected areas) or other signs of post-surgical complications like hypo-or hyperactivity, restlessness, aggressiveness, ataxia, cyanosis, shallow, rapid, or labored breathing, failure to groom, soiled anogenital area, vocalization, circling, head tilt, or hunched posture we will seek veterinary consultation and treat the animals in accordance with the recommendations of the attending veterinarians.

View	Isoflurane (Forane)	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	1-3%	Inhalation	stereotatic surgery and imaging	approx. 30 minutes for surgery	The purpose of the drug is for an inhalation anesthetic delivered in nonrebreathing circuit with a precision vaporizer for extended surgical procedures. We will monitor the efficacy of the anesthetic by assessing the animal reflexes associated with interdigital pinch and eyeblink/palpebral reflex.
View	Ketamine/Xylazine	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	ketamine 100 mg/kg plus xylazine 10 mg/kg	IP	stereotatic surgery	approx. 20 minutes for surgery	Ketamine/Xylazine will be used as an effective injectable anesthetic agent for stereotaxic surgical procedures only. We will monitor the efficacy of the anesthetic by monitoring respiratory rate and efforts and assessing the animal reflexes associated with interdigital pinch and eyeblink/palpebral reflex. In case of unadequate depth of anesthesia, surgical procedure will be paused and 30% or less of the original ketaimine dose will be administered to ensure optimal anesthesia depth. When we using xylazine in rats, and this drug effects will be reversed with Yohimbine (2.1 mg/kg IP) at the conclusion of the procedure to reduce the risk of side effects.
View	Ketoprofen	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	10mg/kg	SQ	Every 12 hours	4 days	Ketoprofen injections will be at 12 hour (or less) intervals for preemptive analgesia and post-op analgesia. All animals will be monitored at regular intervals during the postoperative period following ketoprofen administration. If mice exhibit signs of post-operative complications (i.e., swelling, redness, guarding of the affected areas) or other signs of post-surgical complications like hypo-or hyperactivity, restlessness, aggressiveness, ataxia, cyanosis, shallow, rapid, or labored breathing, failure to groom, soiled anogenital area, vocalization, circling, head tilt, or hunched posture we will seek veterinary consultation and treat the animals in accordance with the recommendations of the attending veterinarians.
View	Meloxicam	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)	5.0mg/kg C	SQ	Once	Pre-emptively	Alternative and given only if Ketoprofen is not available. Given once pre-emptively.

Chemical Agents - Vehicle - Diluent - Solvent

15.2

15.2.1 * **Indicate the type of Chemical Agent you will administer to animals in this study:**

☐ Conventional Chemical Name

☐ Novel Chemical Name or Structure

☑ **Vehicle/Diluent/Solvent**

Vehicle/Diluent/Solvent Details

15.5

15.5.1 * **Please press +Add and complete the chart below for each Vehicle/Diluent/Solvent used.** >>Get Help

View

Agent	PBS
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	5 microlitre/intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Used as solvent
Expected Effect	None

View

Agent	0.5% methylcellulose, 0.2% Tween-80 (MCT)
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	100 ul PO
Frequency/Interval	Daily
Duration	30 days
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Vehicle control
Expected Effect	None

View

Agent	0.5% HPMC, 0.2% Tween in distilled water
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	100 ul PO
Frequency/Interval	Daily
Duration	30 days
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Diluent for Dabrafenib
Expected Effect	No adverse reaction expected

Pharmacological Agent

15.6

15.6.1 * **Please press +Add and complete the chart below for each Pharmacological Agent used.** >>Get Help

View

Agent	Liposomal Suspension
Species	Mouse: BL/6 (6-10 weeks/Male and Female)
Dose/Volume/Route	100-200uL injection volume IP
Frequency/Interval	Every 2 days
Duration	8 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	This is the control for Liposomal Chlodronate treatment
Expected Effect	No side effects, adverse reactions, physiological perturbations expected.

View

Agent	Trametinib
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	25 mg/kg 100 ul PO or 1.5 mg/kg diet dose in formulated chow
Frequency/Interval	Everyday
Duration	6 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Trametinib is a MEK inhibitor that has shown to have great effect in inducing cell death in melanoma cells in-vitro and in-vivo
Expected Effect	None

View

Agent	Ceritinib
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	50 mg/kg 100 ul PO
Frequency/Interval	everyday
Duration	6 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Ceritinib has been shown to induce apoptosis in specific cell lines.
Expected Effect	None.

View

Agent	Dabrafenib
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	30 mg/kg 100 ul PO or 150 mg/kg diet dose in formulated chow
Frequency/Interval	Daily
Duration	6 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Dabrafenib has been show to be efficacious in NRAS mutant melanoma cell lines
Expected Effect	None

View

Agent	Galunisertib (LY2157299)
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	25 mg/kg 100 ul PO or 500 mg/kg diet dose in formulated chow
Frequency/Interval	everyday
Duration	6 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Galunisertib (LY2157299) is a TGF-beta inhibitor. Our preliminary in-vitro data showed that TGF-beta may play a role in the growth of melanoma cells
Expected Effect	None

View

Agent	Liposomal Chlodronate
Species	Mouse: BL/6 (6-10 weeks/Male and Female)
Dose/Volume/Route	1-2mg per 25g body weight in 100-200uL injection volume IP
Frequency/Interval	Every 2 days
Duration	8 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Liposomal Chlodronate suspension is used for depletion of macrophage cells. When animals are treated with liposomal chlodronate, phagocytic cells recognize the liposomes as invading foreign particles and proceed to remove the liposomes from the local tissue or serum via phagocytosis. The liposomes then release clodronate into the cytosol, resulting in cell death.
Expected Effect	No side effects, adverse reactions, physiological perturbations expected. Possible delay of tumor growth/progression.

Tumor Cell Lines

15.9

15.9.1 * **Please press +Add and complete the chart below for each Tumor Cell Line used.** >>Get Help

View

Agent	Fresh CSF-CTCs from Mice
Species	Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female)
Dose/Volume/Route	50,000 cells /5 microlitre/intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Brain tumor formation
Expected Effect	Brain tumor formation

View

Agent	Melanoma cell lines WM1346, WM1366, WM1361A, M245, SKMel103, 1205Lu, M229, M249, WM793, 451Lu, WM164, WM983A, and SM1. We will select two to three lines from the list. label them with GFP-Flu or mCherry-Fluc
Species	Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)
Dose/Volume/Route	50,000 cells /5 microlitre /intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Brain tumor formation
Expected Effect	Brian tumor formation

View

Agent	Breast cancer cell lines TUBO, E0771 and MT901. They will be label with GFP-Flu or mCherry-Fluc (Research for DOD)
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	50,000 cells /5 microlitre /intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Brain tumor formation
Expected Effect	Brain tumor formation

Primary Human Explants - Tissue, Cells or Fluids

15.11

15.11.1 * **Please press +Add and complete the chart below for each Primary Human Explant - Tissue, Cells, or Fluids to be administered to animals.** >>Get Help

View

Agent	Interleukin-2
Species	Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)
Dose/Volume/Route	220,000 IU/100ul/IP
Frequency/Interval	daily
Duration	three days
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Aids in survival and expansion of transferred T cells.
Expected Effect	No untoward effect on animal.

View

Agent	Human CAR-T lymphocytes. Source: buffy coats purchased from Florida Blood Services (St. Petersburg, FL).
Species	Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)
Dose/Volume/Route	1x10e7 cells/100ul/IV or 1x10e5/2ul intracranial in the lateral ventricle through implanted Top Hat/cannula
Frequency/Interval	once IV, 1 to 5 times/intracranial delivery
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	N/A
Purpose of Substance	Regression of tumors.
Expected Effect	No untoward effect on animal.

View

Agent	Fresh CSF-CTCs from melanoma patient with LMDz
Species	Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female)
Dose/Volume/Route	50,000 cells /5 microlitre /intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Brain Tumor Formation
Expected Effect	Brain Tumor Formation

View

Agent	Ex-vivo CSF-CTCs expressing GFP-Flu or mCherry-Flu
Species	Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female)
Dose/Volume/Route	50,000 cells /5 microlitre /intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Brain Tumor Formation
Expected Effect	Brain Tumor Formation

Other Agents

15.12

15.12.1 * **Please press +Add and complete the chart below for other agents used.**

View

Agent	Evans Blue
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	2% solution/ 5 microlitre/Intrathecally (it)
Frequency/Interval	Once
Duration	N/A
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	To ensure that the injections are reaching the intrathecal space
Expected Effect	None

View

Agent	IgG1 isotype control
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	200ug in 200ul via Intraperitoneal (i.p)
Frequency/Interval	Every 5-7 days
Duration	8 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	This is the isotype control for the PD1 antibody
Expected Effect	No side effects, adverse reactions, physiological perturbations expected. Possible delay of tumor growth/progression.

View

Agent	Anti-Her2 Antibody
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	100ug in 200ul via Intraperitoneal (i.p) or 2ug intrathecal (IT)
Frequency/Interval	Once a week
Duration	3 weeks
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Aids Murine Dendritic Cells regression of tumor growth
Expected Effect	No side effects; expected brain metastasis/LMDz regression

View

Agent	Murine Dendritic cells
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	1e6/ 0.05ml/ intratumoral and 1e6/0.1ml/ SC or 1e5/0.004ml Intrathecal
Frequency/Interval	1x per week for intramural and 2x per week for SC or Intrathecal
Duration	3 weeks
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Aids regression of tumor growth
Expected Effect	None side effects; expected brain metastasis/LMDz regression

View

Agent	CKM Adjuvent
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	100ug in 200ul via Intraperitoneal (i.p)
Frequency/Interval	Once a week
Duration	3 weeks
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Aids Murine Dendritic Cells regression of tumor growth
Expected Effect	No side effects; expected brain metastasis/LMDz regression

View

Agent	Anti-Her3 Antibody
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	100ug in 200ul via Intraperitoneal (i.p) or 2ug intrathecal (IT)
Frequency/Interval	Once a week
Duration	3 weeks
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	Aids Murine Dendritic Cells regression of tumor growth
Expected Effect	No side effects; expected brain metastasis/LMDz regression

View

Agent	PD-1 Antibody
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	200ug in 200ul via Intraperitoneal (i.p)
Frequency/Interval	Every 5-7 days
Duration	8 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Aids Murine Dendritic Cells regression of tumor growth
Expected Effect	No side effects; expected brain metastasis/LMDz regression

View

Agent	PD-L1 Antibody
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	100ug in 200ul via Intraperitoneal (i.p)
Frequency/Interval	Once a week
Duration	3 weeks
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	Aids Murine Dendritic Cells regression of tumor growth
Expected Effect	No side effects; expected brain metastasis/LMDz regression

View

Agent	Luciferin
Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: NSG (6-10 weeks/Male and Female) Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Dose/Volume/Route	150 ug per gram body weight
Frequency/Interval	One time
Duration	5-10 min
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	Not available
Purpose of Substance	To measure brain tumor development
Expected Effect	To measure brain tumor development

Substance Administration Final Page

15.13

15.13.1 * **Is there a possibility that any of the test substance(s) could cause more than momentary or slight pain, discomfort, or distress to the animals, either immediately following substance administration, or as a consequence long after administering the test substance(s)?**
● **Yes** ○ No

15.13.1a **If Yes, for each such substance, describe the consequences of administration that have a potential to cause animal discomfort, pain, or distress, and how discomfort, pain or distress will be anticipated, minimized or alleviated (e.g., nude mice administered tumor cells will be humanely euthanatized if induced tumors become >1.0 cm in diameter, or if tumors ulcerate, or if tumors interfere with posture, locomotion or feeding). If discomfort, pain, or distress will not be alleviated, then justify how treatments interfere with the procedures or the interpretation of results:**
The rates of successful inoculation (number of animals in which CSF-CTCs grow/number of animals inoculated). This will be determined on the basis of imaging using the IVIS 200 and on histological evidence of CSF-CTC tumor growth on autopsy. Survival will be measured and determined. 3) Tumors successfully inoculated will be assessed histologically using standards markers to assess their derivation (i.e. melanoma derived v.s. an inflammatory reaction or other process etc.) and characteristics. 4) Tumor growth and distribution within the CSF and CNS will be determined using imaging and histological examination at autopsy.

These endpoints will be assessed as follows. All animals may develop LMDz. Tumors will be monitored weekly by immunofluorescence/luminescence which will be measured using the IVIS 200 in vivo fluorescence and luminescence imaging system. In this way, tumor growth can be quantified based on signal intensity and distribution. Mice will be observed daily for signs of disease or discomfort. Mice will be weighed twice weekly on Monday and Thursday. Mice that lose more than 20% of body weight (rapid weight loss over two to four days; or progressive weight loss over a few weeks), or show signs of morbidity or distress (e.g. hypoactivity, cyanosis, pale mucous membranes, head pressing, head tilt, circling, impaired locomotion, non-purposeful movements, failure to groom, failure to respond to stimuli, isolation from cage mates, shivering, ataxia, shallow, rapid and/or labored breathing, soiled anogenital area, vocalization, lack of inquisitiveness, and/or a hunched posture) will be euthanized. They will be examined grossly and histologically for the extent of tumor in the CNS and in extra-CNS sites if appropriate at autopsy.

15.13.2 * **Are any of the test substances listed above considered a regulated or potentially hazardous material to research or animal care personnel?**
● **Yes** ○ No

15.13.2a **If Yes, (1) list those substances in the space below, (2) describe procedures to ensure these substances and/or animals to which these substances have been administered will be handled safely (e.g., BSL-2, USF Chemical Hygiene Plan, Universal Precautions), and (3) provide an assurance that laboratory and animal care management and staff will be notified when such substances are in use:** >>Get Help
We will CSF-CTCs collected from melanoma leptomeningeal disease patients. Will administer these substances into animals at BSL2 level and after that will house animals at ABSL2. We will update animal care management and staff when these substances are used.

15.13.2b **Please upload any additional information regarding substances to be administered to animals (e.g., characterization of rodent biologics as pathogen-free, a detailed chemical structure for unique test substances that will allow their analysis for potential occupational hazards):**

Name	Modified	Version
There are no items to display		

Surgery

17.1

Section 17 must be completed separately for each species used in surgical procedures. Select a species and all applicable strains/lines involved. If multiple species will be used in surgical procedures, answer "Yes" to 17.1.10 and complete an additional Surgery section.

17.1.1 * **Species:**

Mouse: Athymic NCR nu/nu Strain 490 (6-10 weeks/Male and Female)

Mouse: BALB/cJ (6-10 weeks/Male and Female)

Mouse: BL/6 (6-10 weeks/Male and Female)

Mouse: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (6-8 weeks/15-20gm/M,F)

Mouse: NSG (6-10 weeks/Male and Female)

17.1.2 * **Select all that apply:**
Survival

17.1.2a **Please list personnel that will conduct survival surgical procedures. If delegated to Comparative Medicine surgeons, these surgeons need not be individually named:** >>Get Help

Name	AALAS Aseptic Training	Aseptic Wet Lab Certificate	Link to Profile
Vincent Law	Rodent Aseptic Surgical Technique Training and Exam Completion Certificate(0.01)	VL-Aseptic Surgical Training Wet Lab Certificate.pdf(0.01)	00016893
PI: Peter Forsyth	PF-Aseptic Technique for Rodent Survival Surgery.pdf(0.01) ...		00004562

17.1.3 * **Describe pre-operative medications, including pre-emptive analgesia, tranquilizers, sedatives, pre-anesthetics, and general anesthetics, and their dose and route:** >>Get Help
For general anesthesia we will use Isoflurane (1-3% for 30 minutes). Buprenorphine SR will be given at 0.5-1.5 mg/kg body weight (mice), and Lidocaine block will be given at 5 mg/kg in area of surgery prior to cutting skin. If additional anesthetic is needed, the vaporizer setting should be adjusted accordingly . Body temperature will be maintained with a warm water blanket. Carprofen at 10 mg/kg SC will be administered as a pre-emptive analgesic during the induction phase of anesthesia.

17.1.4 * **Describe pre-operative procedures, including the withholding of food or water, (for what period of time), shaving of hair with enough border to prevent contamination of the operative site, three surgical germicidal scrubs of site, draping with sterile drapes, and the placement of leads, probes, or catheters.** >>Get Help
Pre-operative procedures before surgeries:

I) Preparation of the mouse will be performed in a location remote from the operating area. The surgical site (i.e., dorsal surface of skull) will be clipped with enough border area to keep fur from contaminating the incision site, then it will be scrubbed thrice with a germicidal scrub working from the center of the site toward the periphery, and then rinsed with 70% alcohol. Next, the surgical site will be painted with a tamed dilute iodine solution. Either sterile drapes or a sterile, adhesive-backed plastic drape material (i.e., Bioclusive) is then applied to protect the surgical site from contamination.

17.1.5 * **Describe each surgical procedure in chronological order and in sufficient detail so that the IACUC will be able to determine what is being performed on each animal:** >>Get Help
I) Cisterna Magna (IT) Injections Procedure:

Anesthetized rodent is placed into a small animal stereotaxic with the nose pointed down and positioned to get an approximate 110 degrees –130 degrees “bend” to open up the gap between the skull and C2. Insertion of a small gauge needle (30g mice) into the cisterna magna is performed (the triangular space between the back of the cerebellum and the pyramids, just anterior to the area postrema) and the needle advanced just until cerebral spinal fluid (CSF) is seen within the attached silastic tubing. Tubing is attached in turn to a syringe and 5ul of CSF aspirated, a new syringe containing a replacement volume of fluid (e.g.: Evan’s blue for assessment) is attached and delivered. Two approaches will be modeled; either a percutaneous stick through the skin, or to better visualize blood vessels, via surgical approach and minimal blunt dissection of the muscles just over the cisterna magna. An incision of ~1cm will be made beginning mid-skull and extending dorsally; the muscles will be gently dissected and retracted enough to visualize the dura mater and create a clear window for placement of the needle. The surgical incision will be closed with sterile stainless steel wound clips. Animal will be recovered to assess for obvious signs of injury (e.g.: ataxic, staggering, labored breathing, etc.) for up to 48hrs; animals may be euthanized to assess Evan’s blue placement within the tissue.

Research for DOD
II) Direct Intrathecal (Intraventricular) Delivery of Drugs to the Rodent CNS Procedure:

Implantation of ICV Injectable Port:

Anesthetized mouse is placed into a small animal stereotaxic with the nose in nose cone pointed down and head is secured with ear bars. Wipe the mouse skull clean with sterile cotton swabs, followed by a cotton swab dipped in hydrogen peroxide to enhance bregma. With the curved hemostat, grab the cannula (port), which has a spacer base at the groove where the top meets the pedestal. Move the cannula driver into position and secure into place. Place a single drop of super glue on the spacer base of the cannula. Push the top of the cannula into the driver. Touch the catheter tip to bregma and zero the coordinates on the Digital display and raise the catheter and move 1.1mm laterally to the right and 0.5mm posterior. Hold the skin out of the way with the curved hemostat. Drive the thin metal catheter through the skull until the plastic cannula base is securely pressed against the top of the skull. The metal catheter can be drive directly through the skull in mice due to the relative thin skull. Pull any skin that has glue on it away from the skull with the cannula driver holding the cannula/catheter in place, wait 1-2 minutes for the glue to fully dry. When practicing to assess catheter placement, inject ul volume of Evan’s blue. Wait 2.3 minutes. Sacrifice the mouse and remove the brain. Cut coronally at the catheter site and if the catheter was placed properly in the ventricle, the dye should be perused throughout the mouse ventricular system. Use a cotton swab and press down on the top of the cannula. Clip off the top of the cannula while still pressing down with the cotton swab and try to keep the clippers level so as not to detach the cannula from the skull. If the cannula does come unglued, quickly re-glue and apply pressure with a cotton swab for an additional 2 minutes. Using the 5-0 suture thread, regular hemostat, and forceps, suture along the full opening, paying close attention right over the cannula. Apply a dab of antibiotic ointment over the head and unscrew the ear bars and loosen the nose cone. Remove the mouse form the stereotaxic and place on a warming pad for recovery. Mice will be monitored closely for the duration of recovery and check of obvious signs of injury (ie: ataxic, staggering, labored breathing, etc.) for up to 48 hours; Animals will be euthanized if obvious signs of injury occur.

17.1.6 * **Describe pre- and intra-operative monitoring procedures of depth of anesthesia (e.g., rate & depth of respiration, inter-digital pinch or palpebral blink reflex), and describe all intra-operative medications and/or support:** >>Get Help
I) Depth of anesthesia will be assessed using an interdigital pinch reflex, and by monitoring the depth and rate of respiration. If additional injectable anesthesia is needed, ketamine alone will be administered at 1/3 initial dose. To prevent hypothermia, rodents will be placed on a warm blanket/pad. Subcutaneously administered warmed isotonic fluids will be given at the rate of 10 ml/kg/hr to aid in recovery from the surgery.

17.1.7 * **Describe immediate post-operative monitoring procedures. Animals recovering from general anesthesia must be monitored at least until they are sternally recumbent and capable of purposeful movement.**
I) Mice will be allowed to recover from anesthesia on a warm blanket and observed continuously until recovered as indicated by being sternal and by purposeful movement.

Research for DOD
II) Implantation of ICV Injectable Port Post-operative Care:

1. Monitor the mice daily after the surgery for the first week. If a mouse appears to be in pain or distress, it is recommend providing the mouse with a 10 mg/kg subcutaneous injection of Carprofen once every 24 hr for up to 5 days in order to relieve the pain.

2. If there appears to be an infection, generously apply antibiotic ointment to the area daily and consult an Attending Veterinarian to ensure that the wound properly heals.

3. Remove the 5-0 nylon suture 7-10 days following surgery to prevent irritation from the suture thread.

17.1.8 * **Describe post-operative monitoring and care after animals are returned to housing, including the administration of analgesics, medications, fluids, and any other support methods (dose, route and frequency of post-operative analgesics and medications must be described), and indicate that skin sutures or staples will be removed at approximately 10 - 14 days post-operatively:** >>Get Help
I) Buprenorphine Sustained Release that was given pre-emptively will last up to 72 hours, so additional analgesics are not anticipated to be necessary. However, animals will be checked daily and Meloxicam (or Ketoprofen if Meloxicam is not readily available) will be given if necessary (animals lethargic, not eating, ruffled, etc). Clips will be removed 10-14 days post-operatively. If a surgical site develops signs of post-operative infection (i.e. redness, swelling, tenderness, allodynia, hyperalgesia, hyperpathia, suppuration) or if mice are guarding the affected area, the mouse will be euthanized.

Research for DOD
II) Changing or Removing ICV Injectable Port:

1. The ICV Injectable stays permanently during the trial and if the port is dislodged, affected mouse will be monitored daily for health concern and discomfort. Treatment for that particular animal will be stopped, and euthanized if necessary.

17.1.9 * **Will more than one major survival surgical procedure be performed on a single animal? (Respond “No” if a survival surgical procedure is followed by a non-survival surgical procedure.)** >>Get Help
● **Yes** ○ No

17.1.9a **If Yes, name the procedures and provide scientific rationale/justification:**
Cisterna magna injection (surgical approach) of tumor cells on Day 1. Once brain tumor is developed (usually between 7-12 days), Prior to this period, ICV injectable port will be implanted.

Research for DOD
Cisterna magna injection re-challenge (surgical approach) of tumor cells will only be completed in mice with no detectable tumor mass as evident via IVIS imaging at Day 20. Clinical endpoints will be observed as described throughout course of the study.

17.1.10 **For complicated surgical procedures/design/equipment etc., please upload a detailed diagram or SOPs:**

Name	Modified	Version
There are no items to display		

17.1.11 * **Within this protocol, will another animal species be used in surgical procedures?**
○ Yes ● **No**

Diagnostic Imaging and Microscopy

18.2

18.2.1 * **Select the diagnostic imaging or microscopy procedure(s) (check all that apply):**
Bioluminescence

18.2.2 * **Describe each procedure separately:** >>Get Help
Bioluminescence imaging using the Caliper IVIS 200 or IVIS Spectrum
CTCs and melanoma cell lines tranduced with mCherry-FLuc or GFP-Fluc can be followed via Bioluminescence each week. In this way both tumor cells can be identified NSG mice and Nude rats to determine tumor growth.

Due to auto-fluorescence, hair will be removed from the anatomical regions to be imaged by chemical depilatory (e.g. Nair). Mice will be anesthetized with 2-4% isoflurane, first in an induction chamber. Mice previously inoculated with mCherry-FLuc-transduced cells will be IP injected with 10 ul per g body weight of sterile d-luciferin substrate prepared in PBS at 15 mg/ml (resulting dose 150 ug/ g body weight).

After 5-10 minutes, mice will be transferred to the thermo-regulated, dark chamber of the IVIS located in the SRB vivarium. This complete workstation contains the isoflurane anesthesia system (with induction chamber and oxygen scavenging), temperature controlled (37 degrees C) light-tight animal chamber with anesthesia nosecone manifold, CCD camera, cryogenic unit (for cooling camera), and computer to control and analyze bioluminescent imaging. Up to five mice can be imaged simultaneously.

The system acquires and overlays photographic and luminescent images by measuring photons emitted from luciferase-expressing cells and transmitted through the tissue and analyze them with Living Image software.

Animals will be kept warm under light isoflurane anesthesia throughout the procedure and will feel neither pain nor distress. They will be returned to clean cages after the procedure and allowed to awaken naturally to sternal recumbency and purposeful movement. This noninvasive imaging can be performed repeatedly or sequentially on the same animals and thus allows progressive localization and quantization of tumor growth. Animals are kept warm under light isoflurane anesthesia and will feel no pain or distress. They will be returned to clean cages after the procedure and allowed to awaken naturally to sternal recumbency and purposeful movement.

18.2.3 * **How long will each procedure last? If any procedure is performed more than once, provide frequency and interval:**
Including luciferin injection, isofluorane anesthesia and recovery, about 20-30 minutes each imaging session. We will image animals every week following injection of cells.

18.2.4 * **Describe the methods used to restrain or anesthetize including agent, dose, and route of administration used during imaging:**
During imaging, the animals will be maintained warm within the 37 degree dark chamber and under isofluorane anesthesia via the nosecone manifold. The animals are photographed for the luminescence overlay, thus they can be viewed while in the chamber. In addition, the chamber is opened every few minutes to check and reposition the mice. Following imaging, the mice are returned to clean cages and observed until they revive. Though no separate log of observations is deemed necessary, the Living Image software allows ample annotation of the imaging sessions (animal ID, user ID, date, anatomical view, imaging time and quantitation, etc.).

18.2.5 * **Describe the methods for monitoring the condition of the animals during the procedure and post-procedural period, and whether a log of observations will be kept:**
Animals will be monitored until awake after the procedure for signs of pain or distress, such as gasping, bleeding or frothing at the mouth. If any if these signs are noticed, we will contact the veterinarian right away for assistance. A animal that shows severe signs of respiratory distress will be euthanized immediately.

Other Experimental Procedures

18.8

18.8.1 * **Please press +Add and provide details for each other experimental procedure used:** >>Get Help

View

Species	Mouse: BL/6 (6-10 weeks/Male and Female) Mouse: BALB/cJ (6-10 weeks/Male and Female)
Name of procedure:	Intrathecal (Top Hats) Port Injection
Describe the procedure:	Dosing of animals IT using the ICV (Top Hat) port: Anesthetize the mouse with 2-3% isoflurane, until it shows no signs of righting reflex. In addition, check for tail and/or paw pinch reflex to further ensure the state of anesthesia. The appliance is accessed using a PinPort injector (Instech #PNP3M-50) and a 10ul Hamilton microliter syringe; the pinport injector pierces the septum of the port in a manner that reduces dead space to a minimum and allows controlled injection via motorized syringe pump. Injection of targeted/novel treatment is made over several minutes at specified intervals (days, weekly) for a set duration (weeks/months) while under anesthesia. Once injection is performed, move the mouse back to the cage to recover from anesthesia Tip: a volume between 2-5 µl is optimal as volume less than 2 µl is unreliable and a volume bigger than 5 µl leads to too much pressure.
How long will the procedure last?	3-5 minutes per mouse
Methods to minimize pain:	Mice will be under anesthesia to minimize discomfort.
Describe monitoring:	Mice are monitored during the whole injection process. If anesthesized mice show signs of gasping, bleeding or frothing at the mouth, the treatment will halt and mice will be euthanized immediately.
Describe post-procedure monitoring:	Animals will be monitored until awake after the procedure for signs of pain or distress, such as gasping, bleeding or frothing at the mouth. If any if these signs are noticed, we will contact the veterinarian right away for assistance. A animal that shows severe signs of respiratory distress will be euthanized immediately.
Will a log of observations be kept?	Yes

Instructions for Submission

IS

You have reached the final page of this application.
Submission State: 18. Approved

If your submission state is "Pre Submission", please follow the steps below to submit this application for review.

1. Click the **Finish** button below to exit this form and return to the workspace.
2. Locate the **Submit for Veterinarian Review** activity on the left side of the workspace; click it, and follow any additional instructions to complete your submission.

Please Note: Following Veterinarian Review, the PI will be required to affirm the following upon submission to the IACUC:

- This description is complete and accurate.
- Written IACUC approval will be obtained prior to implementing changes to this protocol.
- Current biomedical supplies will be used.
- Complete animal procedural/surgical/testing records will be maintained.
- Conduct will be in accordance with the PHS Policy, AWR, The Guide, AAALAC Guidelines, DEA Regulations, and IACUC Principles and Procedures.
- Research personnel will abide by all relevant, universal precautions regarding blood-borne pathogens, appropriate biosafety level precautions, radiation safety procedures, and the chemical hygiene plan.
- All vertebrate animals or animal tissues will be acquired by lawful means, used judiciously, and disposed of appropriately.
- Personnel/students are adequately trained and experienced, or supervised to perform these procedures.
- Personnel/students are aware of occupational health & safety practices associated with the use of animals or animal tissues.
- IACUC applications describing teaching or training laboratories must assure the IACUC in writing that they or their designated IACUC-certified staff will continually directly supervise the students, and that prior to the lab commencing, will discuss with the students the potential risks and hazards associated with their involvement in the laboratory involving animals. All attending students will then complete a Student Safety in Teaching Laboratories Involving Animals form, which the PI will sign and provide to Comparative Medicine before the lab commences available at http://www.research.usf.edu/dric/iacuc/occupational-health-safety.asp