VAN ANDEL RESEARCH INSTITUTE
IACUC XENOGRAFT PROTOCOL APPLICATION

This Xenograft Protocol Application (XPA) is a companion form to *Vivarium SOP #6.027- Xenograft & Allografts: The Inoculation and Treatment of Mice*. Only basic xenograft projects can be submitted to the Institutional Animal Care & Use Committee (IACUC) for review using this form. All other xenograft projects must be submitted via the standard IACUC submission process. Basic xenograft projects are defined as projects which include only (but not necessarily all) the following five elements: inoculation/implantation of cell or tissue, blood collection, bone densitometry, ultrasound, and drug therapy.

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WHAT IS THE XPA FORM?

This *Xenograft Protocol Application* (XPA) was designed as a companion form to Vivarium Standard Operating Procedure #6.027. In order to use this form, the PI must be familiar with and comply with the procedures as outlined in the Xenograft SOP. XPA forms are eligible for review by the IACUC Xenograft Subcommittee, following a one-week consideration period by the IACUC. Because the IACUC Xenograft Subcommittee meets once a week, the response time for basic xenograft projects is reduced through the use of the XPA form (to a two-week process from first submission to final approval). In addition, the XPA also incorporates all of the information of the Xenotransplantation Application, so only the XPA form needs to be completed for basic xenograft projects. However, please be aware of these important points:

- Only basic xenograft projects can be submitted to the Institutional Animal Care & Use Committee (IACUC) for review using this form. All other xenograft projects must be submitted via the standard IACUC submission process. Basic xenograft projects are defined as projects which include only (but not necessarily all) the following five elements: inoculation/implantation of cell or tissue, blood collection, bone densitometry, ultrasound, and drug therapy.
- As well as the animal protocol information, this form also incorporates all elements from the Xenotransplantation Application. This means that projects which can be submitted via the XPA form do not need to be submitted separately to the Xenotransplantation Group. This one XPA form covers both needs.

WHAT IS THE SUBMISSION AND REVIEW PROCESS FOR XPA FORMS?

The completed *Xenograft Protocol Application* (XPA) form must be emailed to the IACUC Coordinator (kaye.johnson@vai.org). Some sections (such as *Section VII: Treatment and Dosing Schedules*) may not be applicable to your project. Please note this in the appropriate sections. Once the XPA form has been received, it will be docketed for IACUC review. Once per week, eligible XPA submissions are distributed to the IACUC review committee for consideration for designated review. As long as no member of the IACUC requests full review during the one-week consideration period, the submission will be remanded for designated review by the IACUC Xenograft Subcommittee. Following review, you will be contacted with the protocol disposition (approved, request information, remanded for full IACUC review). If the protocol is approved, the entire XPA form will be forwarded to the Xenotransplantation Group to schedule a Project Start Date. It is optimal to submit the form at least five weeks prior to the Requested Project Start Date (2 weeks for protocol review and 3 weeks to allocate animal housing, schedule tech time, and order animals).

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SECTION I: ADMINISTRATIVE INFORMATION & BILLING DETAILS

Requested Project Start Date:	*5/1/2007*				
Project Title:	*Validation of tumor formation capacity of tissues/cells from knockout Bhd kidney cysts*				
Principal Investigator Name:	Bin Tean Teh				
Principal Investigator Signature: *(This will be obtained after final IACUC approval of the protocol.)*					
PI Address:	Van Andel Research Institute, 333 Bostwick NE, Grand Rapids, MI 49503				
PI Phone:	616-2345296	PI Fax:	616-2345297	PI Email:	bin.teh@vai.org
Project Contact Name:	Jindong Chen				
Contact Address:	Van Andel Research Institute, 333 Bostwick NE, Grand Rapids, MI 49503				
Contact Phone:	616-2345578	Contact Fax:	616-2345579	Contact Email:	jin-dong.chen@vai.org
Study Objectives: *(Briefly explain the aim of the study and why the study is important to human or animal health, the advancement of knowledge or the good of society. The response MUST be in language understandable to a lay person and must clearly indicate your hypothesis.)*	We have previously developed a Bhd kidney-specific knockout mouse model. Since Bhd is a tumor suppressor candidate gene, we expect that the Bhd deficient mice would develop kidney tumors. However, the kidney homozygous deleted mouse can only survive approximately 3 weeks due to kidney failure with polycysts. Pathological analysis showed that hyperplasia in the kidney had occurred, implying great potential to develop kidney tumors if the mice could survive longer period. To test the posibility, we decided to implant the kidney cyst tissue/cells into nude mice by xenograft to see whether any tumor grows in the nude mice.				

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Does this study duplicate previous work of your own or others?	☐ Yes Please provide a justification for the duplication of previous work:	☒ No Please perform a database search to verify that this study is not a duplication of previous work performed by yourself or others. The following information regarding the database search must be provided: Database(s) searched: PubMed Date search(es) performed: 5/01/2007 Dates covered by search(es): 10 years Keywords used in search(es): Bhd, cyst, xenograft In addition, a copy of the search results must be provided to the IACUC Coordinator for placement in the submission file. Please indicate the method you used to provide the results to the IACUC Coordinator: I sent the database search results to the IACUC Coordinator: ☒ As a .pdf file attached to an email sent to kaye.johnson@vai.org ☐ As a printout through interoffice mail to Kaye Johnson, 4th Floor ☐ As a printout placed in Kaye Johnson's mailbox on the 4th Floor.

SECTION II: BILLING DETAILS:

Select one of the following two billing options and provide the appropriate details:

☒ Internal Billing – Van Andel Research Institute account only VARI Account Code: Non-VARI Funding? ☐ Yes ☐ No *(Non-VARI Funding includes all funding external to VARI such as grants, contracts, etc. If you do not have non-VARI Funding, simply indicate "Internal Only" for the next three items)* Funding Source/Agency: Funding/Grant Title: Funding Project Period:	**OR**	☐ External Billing – non-VARI accounts only Institution or Company Name: Institution or Company Address: Billing Reference Code: *This line is for the customer's use to enter any information their Accounts Payable department may require*

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SECTION III: PERSONNEL AUTHORIZED TO PERFORM PROCEDURES

Although the Vivarium SOP (Xenograft & Allografts: The Inoculation and Treatment of Mice) does cite personnel authorized to perform procedures on this protocol (the Vivarium Director, her xenograft technicians, and Dr. Monsma from the Program of Translational Medicine), you have the option of listing additional personnel. You are not required to cite additional personnel if the Vivarium staff will be providing all animal services & interaction for you.

NOTE: If you will be performing surgical inoculation/implantation (such as surgical inoculation to the pancreas as outlined in the SOP), you must provide the name of the surgeon and cite the surgical training and experience.

NAME	DEPARTMENT/LABORATORY	PHONE	FAX	PERFORMING SURGERY?	IF PERFORMING SURGERY, PROVIDE SURGICAL TRAINING/EXPERIENCE
Jindong Chen	Cancer Genetics	616-2345578	616-2345579	Yes ☒ No ☐	
Bin Teh	Cancer Genetics	616-2345296	616-2345297	Yes ☐ No ☒	
Yan Li	Cancer Genetics	616-2345531		Yes ☒ No ☐	
Dan Huang	Cancer Genetics	616-2345683		Yes ☒ No ☐	
				Yes ☐ No ☐	
				Yes ☐ No ☐	

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SECTION IV: EXPERIMENTAL DESIGN

Please outline the experimental design. You may find that bullets, an outline, or a flow chart is useful in explaining your experimental design. The experiment design must allow the IACUC to understand what will happen to each animal (or animal group) from the point of entry into the experiment, through all treatment, to the point of euthanasia or transfer to another animal protocol. In addition, animal usage numbers must be outlined. Explicit details for each of stage of the experiment will be provided in Sections V – IX below.

REMEMBER: Only basic xenograft projects (inoculation/implantation of cell or tissue, blood collection, bone densitometry, ultrasound, and drug therapy) can be submitted through this form. If your study includes any other elements, it must be submitted as a full IACUC protocol. Please contact the IACUC Coordinator if you have any questions.

This project is designed to validate the tumor formation capacity of mouse kidney cyst tissue or cells in nude mice xenograft models. Female athymic nude mice will be used for these studies, typically 4-6 weeks old. The duration of each experiment will be 5-7 weeks for the subcutaneous model and 9-10 weeks for the orthotopic model. All of the experiments will be repeated once to verify findings, for a total of 2 times.

Inoculation/Implantation

Mouse renal cyst cells collected from Bhd knockout mouse kidneys and cultured for 2-3 days in regular DMEM media , will be tested in 2 xenograft models: injected subcutaneously into the right flank of each mouse, or injected orthotopically into the subcapsular region of the left kidney of each mouse through surgery.

For the subcutaneous inoculation, 3 x 10^6-5x 10^6 cells in 0.2ml Hank's balanced salt solution will be used. For the orthotopic inoculation, 1 x 10^6-2 x 10^6 cells in 0.02ml Hank's balanced salt solution will be used. (Inoculation design is based on page 266 of reference 1 below.)

The surgery for the orthotopic implantation will take place on animals that are 4-6 weeks old and the animals will be monitored thereafter for up to 3 months. Monitoring will be through daily observation by Vivarium caretaker staff; they will observe the animals' general health and watching for the criteria for euthanasia. If tumors develop, the tumor volume will be measured twice per week for all models. In addition, we will record bodyweights on a weekly basis. If any animal develops criteria for euthanasia, we will euthanize the animal.

Blood Collection

Blood will be collected if the mouse develop tumors at endpoint. The terminal blood withdrawal will be performed via cardiac puncture (or terminal orbital eyebleed) under isoflurane anesthesia. All of the blood collections will be performed according to Vivarium SOP #6.002 - Blood Collection Techniques in Mice.

Study Endpoint

When the tumors grow to 300 cubic millimeters, the affected mouse will be sacrificed by terminal blood withdrawal. The tumors will be dissected at the terminal day for further studies.

REFERENCES

Reference1 - Inoculation design:

Z. An et al, Development of a high metastatic orthotopic model of human renal cell carcinoma in nude mice: benefits of fragment implantation compared to cell-suspension injection, Clin Exp Metastasis. 1999 May;17(3):265-70. (Inoculation design is modified from page 266.)

SECTION V: INOCULATION / IMPLANTATION

Pathogen test results must be submitted as required by Vivarium SOP #7.003 – Pathogen Testing of Biological Material. Please submit the pathogen test results to the IACUC Coordinator with this completed form. If the biological material will not be pathogen-tested, or the pathogen test results have not yet been received, please indicate that in Section IV – Experimental Design.

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XENOGRAFT GROUPS	**MOUSE STRAIN** *(nu/nu, B6 SCID, C3H SCID, C57BL/6, CB17 SCID, etc.)*	**# OF MICE (F)**	**# OF MICE (M)**	**ANIMAL AGE** *(e.g., 4-6 wks)*	**ANIMAL SOURCE** *VARI Breeding Colony, VARI Repository, Vendor (specify), Other (specify)*	**XENOGRAFT MATERIAL** *List not only the material name, but also the material type and source (e.g., mouse colon carcinoma cells from ATCC).*	**INJECTION / IMPLANTATION METHOD & SITE*** *(e.g., subcutaneous injection between the scapula or surgical implantation into the pancreas.)*	**INJECTATE / IMPLANTATION CONCENTRATION & VOLUME** *(e.g., 1 x 10^6 cells in 100µl of PBS or 1 cubic mm of tissue implanted subcutaneously by trochar.)*
Group #1	athymic nude	20	0	4-6 wks	VARI Repository	Mouse cyst cells from protocol 06-10-028	Subcutaneous Injection in right flank	5 x 10^6 cells in 0.2ml of Hank's balanced salt
Group #2	athymic nude	20	0	4-6 wks	VARI Repository	Mouse cyst cells from protocol 06-10-028	Subcapsular Injection in left kidney	5 x 10^6 cells in 0.2ml of Hank's balanced salt
Group #3	athymic nude	20	0	4-6 wks	VARI Repository	Mouse cyst block from protocol 06-10-028	Subcutaneous Implantation in right flank	8 mm^3 of kidney cyst
Group #4		0	0					
Group #5		0	0					
Group #6		0	0					
Group #7		0	0					
Group #8		0	0					
Group #9		0	0					

***IMPORTANT NOTE:** Only the following methods of inoculation/implantation have been addressed on the companion SOP. Therefore only these methods are eligible for use with this form. If your project calls for a different method of inoculation/implantation, you cannot use this XPA form. Instead, you must submit your project for IACUC review using a standard animal protocol (or amendment to an approved animal protocol, if appropriate). This restriction is due to the fact that the SOP was developed to address common and basic methods of inoculation/implantation to allow an informed, yet accelerated, review.

Methods of Inoculation/Implantation by Stress Category:

Subcutaneous (injection of cells) ..... Category 1 (minimal, transient, or no pain or distress)
Intravenous (injection of cells) ..... Category 1 (minimal, transient, or no pain or distress)
Intraperitoneal (injection of cells) ..... Category 1 (minimal, transient, or no pain or distress)
Intramuscular (injection of cells) ..... Category 1 (minimal, transient, or no pain or distress)
Subcutaneous implantation of solid tumor tissue (see Section 9 of the SOP for details) ..... Category 2 (pain or distress relieved by appropriate measures)

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Surgical inoculation to the pancreas (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the spleen (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the kidney (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the prostate (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the lung (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the breast (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the colon (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)
Surgical inoculation to the stomach (see Section 9 of the SOP for surgery details)..... Category 2 (pain or distress relieved by appropriate measures)

Total Animals by Stress Category:

Using the number of animals which you've entered in the Inoculation/Implantation table above, please determine the total number of animals for each Stress Category and enter the totals below. The form will automatically calculate your total number of animals.

DIRECTIONS: The Stress Category for each animal is based on the highest Stress Category it will undergo during the course of the protocol. Given that this XPA form can only be used with certain implantation and treatment methods, the Category for the mice will always be either Category 1 or Category 2 (projects which include Category 3 animals may not be submitted via this form). Because the treatment options are all Category 1 procedures, the Stress Category will be based on the method of inoculation and any blood collection methods. To simplify, if any animal will undergo non-terminal orbital blood collection, it is automatically classified as Category 2.

Total Number of Animals for Category 1 (minimal, transient, or no pain or distress) 40
Total Number of Animals for Category 2 (pain or distress relieved by appropriate measures) 40
Total Number of Animals for the Protocol (this is calculated by the form; it should match the number of animals cited in your table above) 80

COMMENTS on Section V –
Inoculation / Implantation:

SECTION VI: DATA COLLECTION & TRANSFER

For each of the measurements you need, indicate the frequency of measurement, as well as volume collected (where appropriate). Please note that all tumor measurements will be calculated and recorded in cubic millimeters. In addition, if you will be collecting blood, be sure to specify the blood collection method in the Measurement Notes.

MEASUREMENT TYPE	ONCE A DAY	ONCE A WEEK	TWICE A WEEK	THREE TIMES A WEEK	ONCE A MONTH	SPECIFY OTHER COLLECTION SCHEDULE	BLOOD COLLECTION VOLUME	MEASUREMENT NOTES (Indicate the blood collection method)
Body weight	☐	☒	☐	☐	☐		Not applicable	

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MEASUREMENT TYPE	ONCE A DAY	ONCE A WEEK	TWICE A WEEK	THREE TIMES A WEEK	ONCE A MONTH	SPECIFY OTHER COLLECTION SCHEDULE	BLOOD COLLECTION VOLUME	MEASUREMENT NOTES (Indicate the blood collection method)
Tumor measurement	☐	☒	☐	☐	☐		Not applicable	
Blood (whole)	☐	☒	☐	☐	☐			
Blood serum	☐	☒	☐	☐	☐			
Blood plasma	☐	☒	☐	☐	☐			
Blood hematology	☐	☐	☐	☐	☐			
Blood chemistry	☐	☒	☐	☐	☐			
Bone densitometry	☐	☐	☐	☐	☐			
Ultrasound	☐	☒	☐	☐	☐			
Other Measurement Type:	☐	☐	☐	☐	☐			

Please indicate the preferred frequency for transfer of collected data (only select one option):

- ☒ Transfer data as it is collected
- ☐ Transfer data weekly
- ☐ Transfer data monthly
- ☐ Transfer all data at the end of study only
- ☐ Other data transfer schedule

Data will be collected in an Excel spreadsheet and transferred as an attachment in email.
Please indicate a contact name and email address for receipt of collected data:

Data recipient name: Jindong Chen
Data recipient email: jin-dong.chen@vai.org

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Please indicate the preferred frequency for transfer of collected samples:

- ☒ Transfer samples as they are collected
- ☐ Transfer samples weekly
- ☐ Transfer samples monthly
- ☐ Transfer all samples at the end of study only
- ☐ Other sample transfer schedule

Samples will be shipped via commercial carrier to external clients. Please indicate a contact name, email, and shipping address for receipt of collected samples:

Samples recipient name:

Samples recipient email:

Samples recipient address:

SECTION VII: TREATMENT AND DOSING SCHEDULES

Will you be administering treatment to the xenograft animals?

- ☐ Yes (please complete the treatment table below)
- ☒ No (please skip the rest of Section VII and go directly to Section VIII – Other Procedures)

IMPORTANT NOTE: Be sure to cross-reference the Treatment Groups in the table below to the Inoculation/Implantation Groups cited in the table above in Section V: Inoculation / Implantation. This is essential so that the IACUC reviewers can easily understand which inoculation groups will receive which treatment.

TREATMENT DETAILS: COMPOUND NAME	**TREATMENT COMMENCEMENT & LENGTH** *(for example, begin treatment 3 weeks prior to xenograft and continue for 6 weeks, or begin treatment when tumor reaches 2 cubic millimeters and continue for 2 months)*	**SCHEDULE OF TREATMENT** *(for example, inject twice daily for the duration of the dosing)*	**ROUTE & SITE OF ADMINISTRATION** *(for example, subcutaneous injection in the flank or oral gavage performed according to Vivarium SOP 6.014 - Oral Gavage of Mice)*	**VOLUME & CONCENTRATION** *(for example, 10 mg/kg in 100µl)*	**MSDS SUBMITTED TO VARI IACUC**
					Yes ☐
					Yes ☐
					Yes ☐
					Yes ☐

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					Yes ☐
					Yes ☐
					Yes ☐
					Yes ☐
					Yes ☐

***IMPORTANT NOTE:** Only the following routes of treatment have been addressed on the companion SOP. Therefore only these routes are eligible for use with this form. If your project calls for a different route for administration of treatment, you cannot use this XPA form. Instead, you must submit your project for IACUC review using a standard animal protocol (or amendment to an approved animal protocol, if appropriate). This restriction is due to the fact that the SOP was developed to address common and basic treatment routes to allow an informed, yet accelerated, review.

Treatment Administration Routes by Stress Category:

Subcutaneous injection ..... Category 1 (minimal, transient, or no pain or distress)
Intravenous injection ..... Category 1 (minimal, transient, or no pain or distress)
Intraperitoneal injection ..... Category 1 (minimal, transient, or no pain or distress)
Intramuscular injection ..... Category 1 (minimal, transient, or no pain or distress)
Oral gavage (performed according to Vivarium SOP 6.014 – Oral Gavage of Mice) ..... Category 1 (minimal, transient, or no pain or distress)

COMMENTS on Section VII –
Treatment And Dosing Schedules:

VAN ANDEL RESEARCH INSTITUTE
IACUC XENOGRAFT PROTOCOL APPLICATION

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SECTION VIII: OTHER PROCEDURES

IMPORTANT NOTE: If there any other procedures that need to be incorporated in this xenograft project (other than inoculation/implantation of cell or tissue, blood collection, bone densitometry, ultrasound, and drug therapy), then this project does not meet the criteria for a basic xenograft protocol. Please submit this work for IACUC review as a standard animal protocol (or amendment to an approved animal protocol, if appropriate). In addition, once IACUC approval has been received, the work will also need to be submitted to the Xenotransplantation group on a Xenotransplantation application (the IACUC review addresses animal care and use concerns and the Xenotransplantation group handles scheduling and performing the work itself). Only basic xenograft projects are eligible to be submitted on the Xenograft Protocol Application (which combines IACUC Review with the Xenotransplantation group application).

SECTION IX: STUDY ENDPOINT AND NECROPSY

What determines the experimental endpoint*?

☒ Euthanize **individual animals** based on tumor size.
Tumor size: 300 cubic millimeters

☐ Euthanize the **entire animal group** based on tumor size
(as soon as the first animal reaches maximum tumor size).
Tumor size:
Please indicate which animal group(s) will be euthanized under this endpoint:

☐ Euthanize the **entire study** based on tumor size
(as soon as the first animal reaches maximum tumor size).
Tumor size:

☐ Euthanize a scheduled number of days post-injection/implantation.
Number of days:

☐ Other experimental endpoint
Please provide full details of the experimental endpoint(s). Be sure to indicate what is the determining factor for the endpoint (time, specific symptomology, etc.) and indicate which group(s) will be euthanized according to which endpoint(s).

Please note that individual animals may need to be euthanized prior to reaching the designated experimental endpoint if they meet the criteria for euthanasia as outlined in Vivarium SOP #6.027 - Xenograft & Allografts: The Inoculation and Treatment of Mice. In this case, the Vivarium staff will phone and email the Principal

VAN ANDEL RESEARCH INSTITUTE
IACUC XENOGRAFT PROTOCOL APPLICATION

This Xenograft Protocol Application (XPA) is a companion form to *Vivarium SOP #6.027- Xenograft & Allografts: The Inoculation and Treatment of Mice*. Only basic xenograft projects can be submitted to the Institutional Animal Care & Use Committee (IACUC) for review using this form. All other xenograft projects must be submitted via the standard IACUC submission process. Basic xenograft projects are defined as projects which include only (but not necessarily all) the following five elements: inoculation/implantation of cell or tissue, blood collection, bone densitometry, ultrasound, and drug therapy.

For Administrative Use Only

IACUC Protocol Number: _____
Protocol Approval Date: _____
Protocol Expiration Date: _____
Project Initiation Date: _____
Project Completion Date: _____
Total Tech Cost: _____
Total Care Days: _____

Investigator and/or designated contact person prior to euthanizing the animal. However, if the PI and/or designated contact person is unavailable, the animal will be euthanized and necropsy performed as indicated on this form.

Select which necropsy procedures you want performed.
Please note that all terminal blood collections will be performed according to Vivarium SOP #6.002 – Blood Collection Techniques in Mice.

- ☒ Digital photos
- ☐ Body weight
- ☒ Blood collection – whole
  Volume to be collected:
  Method of collection: ☒ Orbital exsanguination (terminal) ☐ Cardiac puncture (terminal)
- ☐ Blood collection – plasma
  Volume to be collected:
  Method of collection: ☐ Orbital exsanguination (terminal) ☐ Cardiac puncture (terminal)
- ☐ Blood collection – serum
  Volume to be collected:
  Method of collection: ☐ Orbital exsanguination (terminal) ☐ Cardiac puncture (terminal)
- ☒ Blood chemistry
  (Unless otherwise specified above, blood will be collected by orbital exsanguination (terminal) and the maximum available volume will be obtained.)
- ☐ Blood hematology
  (Unless otherwise specified above, blood will be collected by orbital exsanguination (terminal) and the maximum available volume will be obtained.)
- ☐ Lung inflations
- ☐ Fixative perfusion
- ☒ Tissues harvested:
  Specify the tissues to be harvested and indicate the preservation method:

TISSUES TO BE HARVESTED	FROZEN	FORMALIN FIXED	FRESH	OTHER / NOTES
Tumors	☒	☒	☒	
	☐	☐	☐	
	☐	☐	☐	

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TISSUES TO BE HARVESTED	FROZEN	FORMALIN FIXED	FRESH	OTHER / NOTES
	☐	☐	☐	
	☐	☐	☐	
	☐	☐	☐	
	☐	☐	☐	
	☐	☐	☐	
	☐	☐	☐	

COMMENTS on Section IX –
Study Endpoint And Necropsy:

SECTION X: CONTACT INFORMATION

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