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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IACUC Protocol Number: XPA-07-07-020
Protocol Approval Date: July 23, 2007
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Total Care Days:

NOTE: INSTRUCTIONS ARE LOCATED ON THE LAST PAGE OF THIS FORM.

SECTION I: ADMINISTRATIVE INFORMATION & BILLING DETAILS

Requested Project Start Date:	*7/1/2007*				
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.)*	**Signing Certification:** By signing, I certify that the work will be done in accordance with this Xenograft Protocol Application and the related Vivarium SOP #6.027.				
PI Address:	Van Andel Research Institute, 333 Bostwick Avenue NE, Grand Rapids, MI 49503				
PI Phone:	616.234.5296	PI Fax:	616.234.5297	PI Email:	Bin.Teh@vai.org
Project Contact Name:	Jindong Chen				
Contact Address:	Van Andel Research Institute, 333 Bostwick Avenue NE, Grand Rapids, MI 49503				
Contact Phone:	616.234.5578	Contact Fax:	616.234.5579	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 (a gene related to the Birt-Hogg-Dube syndrome) kidney-specific knockout mouse model which is a mouse that does not have the Bhd gene in the kidney. Since Bhd is a tumor suppressor candidate gene, we expect that the Bhd-deficient mice would develop kidney tumors. However, these mice with the deletion of the Bhd gene can only survive for approximately 3 weeks due to kidney failure with polycysts which may not allow enough time for the mice to generate phenotypes like tumor formation. Analysis showed that hyperplasia (proliferation of cells within an organ or tissue beyond that which is ordinarily seen) in the kidney had occurred, implying great potential to develop kidney tumors if the mice could survive for longer periods. To test the possibility, we decided to implant the kidney cyst tissue/cells from the Bhd-deficient homozygous animals into nude mice by xenograft to see whether any tumors will grow in the nude mice. This study is important because it can tell us whether Bhd-deficient kidney cysts or cyst cells can result in tumor formation in xenograft mice, which will demonstrate that Bhd is a tumor suppressor and increase our understanding of BHD-related diseases.				

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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: 6/26/07 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. Comments:

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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: 10301
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:
PI on Grant/Contract:
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

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.

The completed Indication of Roles form for this submission has been emailed to the IACUC Coordinator (kaye.johnson@vai.org): ☒ Yes

NAME	DEPARTMENT/LABORATORY	PHONE	FAX	PERFORMING SURGERY?	IF PERFORMING SURGERY, PROVIDE SURGICAL TRAINING/EXPERIENCE
Bin Tean Teh	Cancer Genetics	616.234.5296	616.234.5297	Yes ☐ No ☒	
Jindong Chen	Cancer Genetics	616.234.5578	616.234.5579	Yes ☒ No ☐	May perform surgery after being instructed by Dr. Huang.

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NAME	DEPARTMENT/LABORATORY	PHONE	FAX	PERFORMING SURGERY?	IF PERFORMING SURGERY, PROVIDE SURGICAL TRAINING/EXPERIENCE
Yan Li	Cancer Genetics	616.234.5531		Yes ☒ No ☐	Dr. Li was trained in subcutaneous tissue implantation at the National Pharmaceutical Institute of China. She has 14 years experience in xenografts.
Dan Huang	Cancer Genetics	616.234.5683		Yes ☒ No ☐	Dr. Huang was trained in subcutaneous inolculation and renal subcapsular inoculation at the National Pharmaceutical Institute of China. She received refresher training from Dr. Miles Qian at the VARI. She has 6 years of xenograft experience.
				Yes ☐ No ☐	
				Yes ☐ No ☐	
				Yes ☐ No ☐	
				Yes ☐ No ☐	

SECTION IV: EXPERIMENTAL DESIGN

Are any of the materials used in this protocol (either for xenograft or for treatment) of human origin?

☒ No ☐ Yes (Please provide a copy of the IRB approval or exemption letter to the IACUC Coordinator.)

Are any of the materials used in this protocol (either for xenograft or for treatment) of non-human origin or of human origin that has been passaged through mice?

☐ No ☒ Yes (Please provide a copy of the pathogen-test results to the IACUC Coordinator, or indicate in the Experimental Design that the material has not yet been tested.)

Are the materials used in this protocol for inoculation sterile or attenuated?

☒ No ☐ Yes Comments:

Are the materials used in this protocol for treatment sterile or attenuated?

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☐ No ☐ Yes Comments: Not Applicable.

NOTE: The tissue and cells used for implantation/inoculation in this protocol are harvested from Dr. Bin Teh's protocol #06-10-028, which is in the barrier portion of the Vivarium. Therefore, no pathogen-test results are needed.

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.

According to Vivarium SOP #6.027, all injections will be performed with a 27 gauge needle, with the exception of surgical inoculations. The needle gauge for each type of surgical inoculation is specified within the surgery text in Section 9 of the SOP. If your study will use a different needle size than specified in the SOP, it must be stipulated in the experimental design below.

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 models 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.

We will set up three groups: subcutaneous cell inoculation group; subcapsular cell inoculation group; subcutaneous tissue implantation group. Each group needs 20 mice and each experiment will be repeated once. From our experience with previous studies using these models, 20 mice have generated the optimum results. The total number of mice needed are calculated below:

3 models x 20 mice per group x 2 times (one plus repeat) = 120 mice + 10% increase for death, error, etc. = 132 mice

Mouse renal cyst cells, collected post-mortem from Bhd knockout mouse kidneys on Dr. Bin Teh's protocol #06-10-028 and cultured for 2 - 3 days in regular Dulbecco's Modified Eagle Medium (DMEM), will be tested in three xenograft models: 1) injected subcutaneously into the right flank of each mouse, 2) injected orthotopically into the subcapsular region of the left kidney of each mouse through surgery, 3) mouse renal cyst tissue blocks (0.3mm x 0.3mm x 0.3mm) will be subcutaneously implanted in the right flank of each mouse following the Vivarium SOP #6.027 – Xenograft & Allografts: The Inoculation and Treatment of Mice.

These experiments will take place on animals that are 4-6 weeks old. Monitoring will be through daily observation of all the animals by Vivarium caretaker staff for up to 3 months. They will observe the animals' general health and watch for criteria for euthanasia. In addition, we will record bodyweights for all the animals on a weekly basis. If any animal develops any other criteria, other than tumor size limit, for euthanasia as specified in the Vivarium SOP #6.031 – Euthanasia of Mice, we will euthanize the animal via CO2. At the end of 3 months, all of the animals (with tumors or not) will be sacrificed by CO2.

Group #1 – Subcutaneous injection in the right flank

For this group, 5 x 10^6 cells in 0.2 ml Hank's balance salt solution (HBSS) will be used.

If these mice develop tumors, the tumor volume will be measure by calipers twice per week. If the tumors grow to 2500 cubic millimeters, the affected mouse will be sacrificed by terminal blood withdrawal. The tumors will be dissected at the terminal day for further studies. Mice which do not develop tumors under the above limit size will be euthanized by CO2 at 3 months post-inoculation. If no tumors grow, the mice will also be euthanized by CO2 at the endpoint.

If the mice develop tumors, blood will be collected weekly up to 3 months. The weekly survival blood collection will be performed by retro-orbital bleed. The terminal blood withdrawal will be performed via cardiac terminal puncture or orbital exsanguinations under isofluane anesthesia. All of the blood collections will be performed according to Vivarium SOP #6.002 – Blood Collection Techniques in Mice.

Group #2 – Orthotopic injection into the subcapsular region of the left kidney

For this group, 2 x 10^6 cells in 0.02ml HBSS will be used (Inoculation design is based on page 266 of reference 1 below).

For the orthotopic inoculation group, the tumor size will be monitored once a week by ultrasound. Ultrasound will be performed according to Vivarium SOP #6.011 – Ultrasound of Mice. If 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. Mice which do not develop tumors under the above limit size will be euthanized by CO2 at 3 months post-inoculation. If no tumors grow, the mice will also be euthanized by CO2 at the endpoint.

If the mice develop tumors, blood will be collected weekly up to 3 months. The weekly survival blood collection will be performed by retro-orbital bleed. The terminal blood withdrawal will be performed via cardiac terminal puncture or orbital exsanguinations under isofluane anesthesia. All of the blood collections will be performed according to Vivarium SOP #6.002 – Blood Collection Techniques in Mice.

Group #3 – Subcutaneous implantation in the right flank

For this group, we will implant mouse renal cyst tissue blocks (0.3mm x 0.3mm x0.3mm) according to the Vivarium SOP #6.027 – Xenografts & Allografts: The Inoculation and Treatment of Mice.

If these mice develop tumors, the tumor volume will be measure by calipers twice per week. If the tumors grow to 2500 cubic millimeters, the affected mouse will be sacrificed by terminal blood withdrawal. The tumors will be dissected at the terminal day for further studies. Mice which do not develop tumors under the

above limit size will be euthanized by CO2 at 3 months post-inoculation. If no tumors grow, the mice will also be euthanized by CO2 at the endpoint.

If the mice develop tumors, blood will be collected weekly up to 3 months. The weekly survival blood collection will be performed by retro-orbital bleed. The terminal blood withdrawal will be performed via cardiac terminal puncture or orbital exsanguinations under isofluane anesthesia. All of the blood collections will be performed according to Vivarium SOP #6.002 – Blood Collection Techniques in Mice.

References:

1. Inoculatation 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):265-270. (Inoculation design is modified from page 266.)

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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.

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	44	0	4-6 wks	VARI Breeding Colony	Mouse cyst cells from Dr. Bin Teh's 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	44	0	4-6 wks	VARI Breeding Colony	Mouse cyst cells from Dr. Bin Teh's protocol #06-10-028	Subcapsular injection in left kidney	2 x 10^6 cells in 0.02ml of Hank's balanced salt
Group #3	athymic nude	44	0	4-6 wks	VARI Breeding Colony	Mouse cyst blocks from Dr. Bin Teh's protocol #06-10-028	Subcutaneous implantation in right flank	mouse renal cyst tissue blocks (0.3mm x 0.3mm x0.3mm)
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.

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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)
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)	0
Total Number of Animals for Category 2 (pain or distress relieved by appropriate measures)	132
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)	**132**

COMMENTS on Section V –
Inoculation / Implantation:

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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	COLLECTION VOLUME OF WHOLEBLOOD (for plasma, serum etc.)	MEASUREMENT NOTES (Indicate the blood collection method)
Body weight	☐	☒	☐	☐	☐		Not applicable	
Tumor measurement	☐	☒	☐	☐	☐		Not applicable	
Blood (whole)	☐	☒	☐	☐	☐		200 ul	Retro-orbital for survival bleeds and orbital exsanguination for terminal bleeds
Blood serum	☐	☒	☐	☐	☐		30 ul serum from 200 ul whole blood	Retro-orbital for survival bleeds and orbital exsanguination for terminal bleeds
Blood plasma	☐	☒	☐	☐	☐		30 ul plasma from 200 ul whole blood	Retro-orbital for survival bleeds and orbital exsanguination for terminal bleeds
Blood hematology	☐	☐	☐	☐	☐			
Blood chemistry	☐	☐	☐	☐	☐			
Bone densitometry	☐	☐	☐	☐	☐			
Ultrasound	☐	☒	☐	☐	☐			Group #2 - Orthotopic injection into the subcapsular region of the left kidney

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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	COLLECTION VOLUME OF WHOLEBLOOD (for plasma, serum etc.)	MEASUREMENT NOTES (Indicate the blood collection method)
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

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

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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: Jindong Chen
Samples recipient email: jin-dong.chen@vai.org
Samples recipient address: Van Andel Research Institute, 333 Bostwick Avenue NE, Grand Rapids, MI 49503

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 ☐
					Yes ☐
					Yes ☐
					Yes ☐
					Yes ☐
					Yes ☐

VAN ANDEL RESEARCH INSTITUTE
IACUC XENOGRAFT PROTOCOL APPLICATION

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For Administrative Use Only

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***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:

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:

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☐ 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).

If the tumors grow to 300 cubic millimeters (for orthotopic inoculation group) or 2500 cubic millimeter (for subcutaneous inoculation and implantation groups) within three months, the affected mouse will be sacrificed by terminal blood withdrawal. The tumors will be dissected at the terminal day for further studies. Mice which do not develop tumors under the above limit size will be euthanized by CO2 at 3 months post-inoculation. If no tumors grow, the mice will also be euthanized by CO2 at the 3 months.

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 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: 200ul is required for analysis, but maximun available will be withdrawn. NOTE: Method of collection will be either orbital exsanguinations or cardiac puncture.

Method of collection: ☒ Orbital exsanguination (terminal) ☒ Cardiac puncture (terminal)

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☐ 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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COMMENTS on Section IX –
Study Endpoint And Necropsy:

SECTION X: CONTACT INFORMATION

Van Andel Research Institute
333 Bostwick Avenue NE
Grand Rapids, MI 49503

Institutional Animal Care & Use Committee (IACUC):
Ms. Kaye Johnson
IACUC Coordinator
Email: kaye.johnson@vai.org
Phone: (616) 234-5702
Fax: (616) 234-5703

Vivarium:
Ms. Bryn Eagleson
Vivarium Director
Email: bryn.eagleson@vai.orgb
Phone: (616) 234-5260
Fax: (616) 234-5261

Program of Translational Medicine:
Dr. David Monsma
Research Scientist
Email: david.monsma@vai.org
Phone: (616) 234-5696
Fax: (616) 234-5697

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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).