Eli Lilly EPA MTA #730-13

MATERIALS TRANSFER AGREEMENT

Provider: Environmental Protection Agency ("EPA")

Recipient: Eli Lilly and Company ("Lilly")

RECITALS

Lilly is engaged in the research, development, manufacture and marketing of pharmaceutical products and is interested in further development of compounds suitable for use as pharmaceutical products.

EPA is willing to provide Research Material (described in Appendix A) to conduct the Research Project.

Lilly and EPA are interested in publishing the data and results of the Research Project.

1. Provider agrees to transfer Research Material to Recipient.

Specified chemicals and/or supporting ToxCast testing data for chemicals identified in a vascular disruption model.

2. This Research Material may not be used in human subjects. The Research Material will be used only for research purposes by Recipient in its laboratories, for the research project described below, under suitable containment conditions. This Research Material will not be used for production or sale, for which a commercialization license may be required. Recipient agrees to comply with all Federal rules and regulations applicable to the Research Project and the handling of the Research Material.

3. Does the Research Material include specimens or data derived or collected from human subjects?
_____ Yes – Go to item #3(a).
__X__ No – Skip to item #4.

3(a). Does the Research Material include specimens or data derived or collected from fetuses, children, pregnant women, or nursing women?
_____ Yes
_____ No

3(b). Was the Research Material obtained under a protocol that was reviewed and approved by an Institutional Review Board (IRB) that operated in accordance with the

requirements of EPA Regulation 40 CFR 26, HHS Regulation 45 CFR 46, or any other Federal Regulation for the protection of human research subjects?
_____ Yes (Please indicate the applicable Regulation here _____ and provide copies of the protocol and IRB approval documents.)
_____ No (Please provide explanation with documentary support as appropriate.)

3(c). Can the Provider of the Research Material identify the subjects directly or through identifiers (codes) linked to the subjects?
_____ Yes – The Recipient's use of the Research Material may be human subject's research subject to 40 CFR 26. Go to item #3(d).
_____ No – The Recipient's use of the Research Material is not human subjects research subject to 40 CFR 26. Skip to item #4.

3(d). Is the Provider of the Research Material prohibited by this agreement from releasing information to the Recipient that might allow the identification of any of the subjects, including but not limited to the key to any existing code?
_____ Yes – The Recipient's use of the Research Material is not human subjects research subject to 40 CFR 26. Skip to item #4.
_____ No – The Recipient's use of the Research Material may be human subjects research subject to 40 CFR 26. Go to item #3(e).

3(e). Is the Research Material publicly available?
_____ Yes – The Recipient's use of the Research Material is human subjects research that is exempt from 40 CFR 26.
_____ No – The Recipient's use of the Research Material is human subjects research that may be subject to 40 CFR 26 and must be further evaluated accordingly by the EPA Human Subjects Review Official.

4. This Research Material will be used by Recipient solely in connection with the research project ("Research Project") described in the attached Appendix A.

5. In all oral presentations or written publications concerning the Research Project, each party will acknowledge the other party's contribution unless requested otherwise. To the extent permitted by law, the parties agree to treat as confidential, any information about the Research Material or Research Project that is labeled as confidential or proprietary ("Confidential Information") for a period of five (5) years from the date of its disclosure to the receiving party by the disclosing party. The foregoing shall not apply to Confidential Information that: was known to receiving party prior to the date of disclosure; is or becomes publicly available or which is disclosed to the receiving party without a confidentiality obligation; is developed by the receiving party without the use of such Confidential Information disclosed by the disclosing party; or is required to be disclosed by law. It is the intent that one or more of the parties will publish or otherwise publicly disclose the results of the Research Project Such public disclosure may be made only after the parties have had an opportunity to review the proposed disclosure to

determine if it includes any Confidential information or patentable subject matter. Either party may delay such publication for up to 90 days to remove its Confidential Information or address patentable subject matter. Any oral disclosures from Provider to Recipient which Provider wishes to be treated as confidential shall be identified as Confidential at the time of the disclosure and by written notice delivered to Recipient within thirty (30) days.

6. This Research Material represents a significant investment on the part of Provider and is considered proprietary to Provider. Recipient therefore agrees to retain control over this Research Material and further agrees not to transfer the Research Material to other people not under its direct supervision without advance written approval of Provider. Provider reserves the right to distribute the Research Material to others and to use it for its own purposes. When the research project is completed, the Research Material will be disposed of by Recipient.

7. This Research Material is provided as a service to the research community. It is being supplied to Recipient with no warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose. Provider makes no representations that the use of the Research Material will not infringe any patent or proprietary rights of third parties.

8. While it is the Party's intent to publish the data and results of the Research Project, the Parties non-the-less acknowledge the possibility that Inventions may be made during the term of the Research Project. Inventorship will be determined in accordance with applicable U.S. laws and regulations. The term "made", as used in reference to any invention herein, means the conception or first actual reduction to practice of such invention.

Inventions made in the course of the Research Project will be owned by the Party employing the inventor or inventors. Inventions that are invented jointly by employees of both Parties will be owned jointly.

Each Party will report to the other Party, in writing, all Inventions made during the Research Project no later than 3 months from the time the invention is disclosed to a Party by its Investigator. The reports will be written in sufficient detail to determine inventorship and will be treated as Confidential Information in accordance with Section 5. The Parties will confer with each other regarding a patent filing strategy for jointly made Inventions. If either Party files a patent application on a jointly made Invention, then the filing Party will include a statement in the patent application that clearly identifies the Parties and states that the Invention was made jointly under this Agreement.

9. Section 5 above not with-standing, when Provider is the EPA: Recipient agrees not to claim, infer, or imply endorsement by the Government of the United States of America (hereinafter referred to as "Government") of the Research Project, the institution or personnel conducting the Research Project or any resulting product(s). Except as required by law or governmental regulation, neither party shall release any information to any third party with respect to the terms of this Agreement without the prior written consent of the other. Recipient agrees to hold the

Government harmless and to indemnify the Government for all liabilities, demands, damages, expenses and losses ("Claims") arising out of Recipient's use for any purpose of the Research Material, except such Claims resulting from the gross negligence or willful misconduct of the Government.

10. Provider will not be liable to EPA for any claims or damages arising from EPA's use of the data and results of the Research Project.

11. The Parties agree that this Agreement will be effective for 1 year from the date of the last authorized signature below and may be extended as mutually agreed by the Parties in a written amendment to this Agreement. This Agreement will terminate upon the mutual agreement of the Parties in writing. This Agreement will terminate in 30 days after either Party receives written notice of the other Party's desire to terminate this Agreement for any reason, including breach. Upon termination or expiration, following the written request of the Provider, Recipient shall destroy all unused portions of the Research Materials.

12. Will EPA develop any products or services from information or materials provided by the Recipient?

____ Yes – go to item A

__X_ No – skip to #13 (next clause)

Item A: The EPA has a long history of applying principles of quality assurance/quality control to all technical work conducted by or for the Agency (CIO 2106: USEPA Quality Policy). Given EPA is receiving metabolomics and screening data and will use the metabolomics and screening data for Agency purposes, the Recipient is required to provide EPA with documentation such as a quality manual, describing their organization's quality system. In lieu of such documentation, Standard Operating Protocols for compound handling and the assays performed are acceptable or documentation showing third party accreditation to a relevant standard and scope is also acceptable for documenting an organization's quality system. EPA requirements for quality management plans can be found at this URL:
http://www.epa.gov/quality/qa_docs.html

13. All notices pertaining to or required by this Agreement shall be in writing and shall be signed by an authorized representative and shall be delivered by hand (including private courier mail service) or sent by certified mail, return receipt requested, with postage prepaid, addressed as follows:

Provider's Administrative Contact Information:

Rebecca Clausen
National Center for Computational Toxicology (NCCT)
US EPA
109 TW Alexander (MD-B-205-01)
Research Triangle Park, NC 27711
For commercial courier address use:
4930 Old Page Rd.
Durham, NC 27703
919-541-3002
clausen.rebecca@epa.gov

Recipient's Contact Information:

Dorothy Ryan Clippert

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DC 1117, Indianapolis, IN 46285
317-277-5972 (ph)
317-276-0894 (fax)
Clippert_dorothy@lilly.com

14. Paragraphs 2, 7, 9 and 10 shall survive termination.

15. This Agreement shall be construed in accordance with law as applied by the Federal courts in the District of Columbia.

16. The undersigned Provider and Recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.

17. This agreement shall enter into force as of the date of the last signature of the parties and shall remain in effect for one year from said date.

APPENDIX A

Proposal: Testing putative Vascular Disruptor Compounds (pVDCs) in functional angiogenesis assays

Hypothesis: First-generation predictive models for prenatal developmental toxicity built from the ToxCast data revealed a complex web of biological processes with many connections to vasculogenesis and angiogenesis (Kleinstreuer *et al.*, 2011; Sipes *et al.*, 2011). A body of literature exists for susceptibility and vulnerability of embryonic vascular development to diverse drugs and chemicals such as thalidomide, estrogens, endothelins, dioxin, retinoids, cigarette smoke, and metals (Knudsen and Kleinstreuer, 2012). This frames the hypothesis that chemical disruption of embryonic vascular development is an adverse outcome pathway (AOP) of potential significance for predictive toxicology and risk assessment.

Background: The cardiovascular system is the first functional organ to develop in the mammalian embryo. A myriad of signals and genes control embryonic vascular development, including: local growth factors and cytokines such as VEGF-A and TGF-beta, components in the plasminogen activator system, and chemotactic chemokines. The conceptual AOP framework is intended to explicitly translate information on molecular and cellular responses into endpoints meaningful to risk assessment (e.g., development, reproduction, survival). An AOP delineates the documented, plausible, and testable processes by which a chemical induces molecular perturbations and the associated biological responses that describe how the molecular perturbations cause effects at the subcellular, cellular, tissue, organ, whole animal, and population levels of observation. This concept requires an anchor to a molecular initiating event (MIE) and an adverse outcome with significance to risk assessment (Ankley *et al.*, 2010). To develop an AOP for embryonic vascular disruption (Figure 1), we searched the gene ontology (GO) and mammalian phenotype (MP) browsers of the Mouse Genome Informatics database (http://www.informatics.jax.org/) for terms affiliated with the disruption of vascular development. Terms for abnormal vasculogenesis [MP:0001622; 72 genotypes, 73 annotations] and abnormal angiogenesis [MP:0000260; 610 genotypes, 894 annotations] were captured into a table and then linked to ToxCast assays. This list had 65 target genes with bona fide roles in vasculogenesis or angiogenesis, 50 of which had evidence of abnormal embryonic vascular development based on genetic mouse models (Knudsen and Kleinstreuer, 2012).

pVDCs: Disruption of embryonic vascular development may be part of an AOP that can be applied to predictive toxicology and mechanistic modeling of prenatal developmental toxicity. As such, an AOP perspective of embryonic vascular development can help identify useful information for assessing adverse outcomes relevant to risk assessment and efficient use of resources for validation. We define the term 'putative vascular disruptor compounds' (pVDCs) as broadly descriptive of any drug or chemical that alters vasculogenesis or angiogenesis [Kleinstreuer et al. 2011]. The MIE of a pVDC may be direct (e.g., perturbation of VEGF signaling) or indirect (e.g., hypoxia-induced VEGF signals); the pathway may be EC-specific (e.g., altered EC tip cell exploratory behavior) or not (e.g., disruption of branching morphogenesis); the target system may be the embryo, placenta or mother; and the adverse outcome may by pleiotropic (low birth weight, functional deficits, malformations, embryolethality). The pVDCs identified for this study include common environmental chemicals such as pesticides and anti-microbials, as well as

pharmaceuticals and consumer product ingredients. There is significant/complete overlap with chemical lists TSCA21/EDSP21, identified as regulatory priorities by EPA.

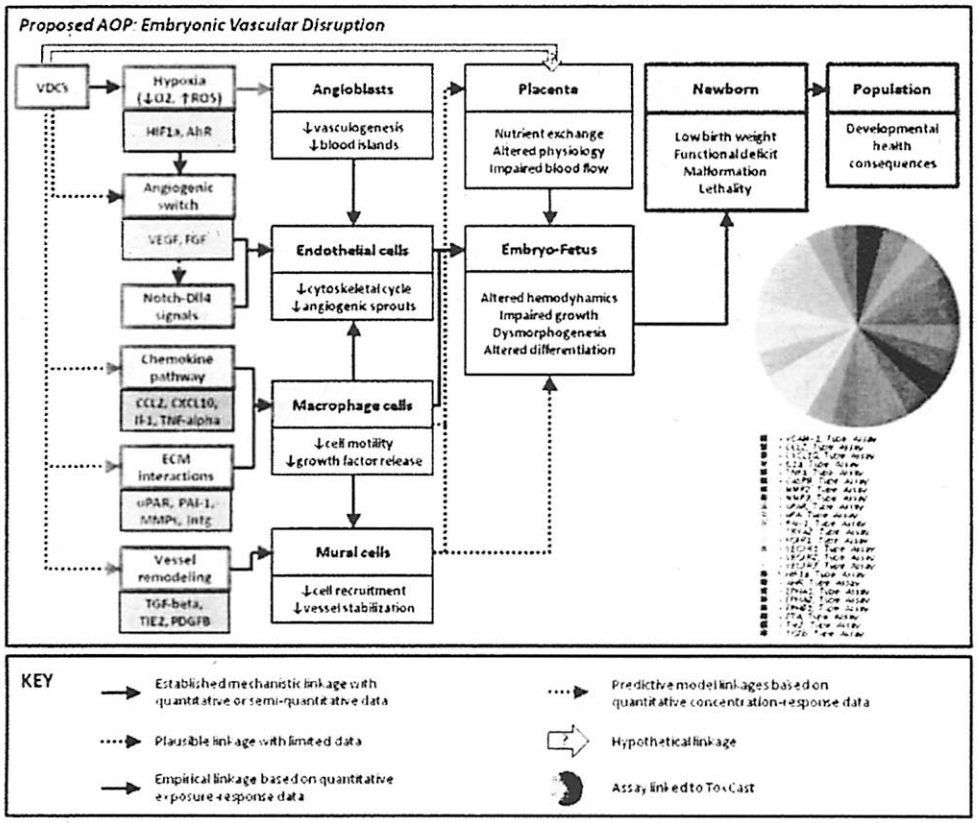

Figure 1. Proposed AOP prototype for embryonic vascular disruption. *The conceptual prototype model is built incorporating ToxCast HTS data into the AOP schema. Anchor 1 (red boxes on the left side) address chemical properties of the toxicant and nature of macromolecular interactions. Anchor 2 (red boxes on the right) refer to relevant organism responses and community-level population responses. The middle columns address cellular and organ responses. The color wheel indicates ToxPi sectors for chemical prioritization (shown here without data applied). The 25 assays shown are those which had evidence of abnormal embryonic vascular development based on genetic mouse models and mapped to previously-identified critical pathways (hypoxia/growth factor signaling, chemokine networks, ECM interactions and vessel remodeling/stabilization); they are color-coded as such in the ToxPi schem; from (Knudsen and Kleinstreuer, 2012).*

Model validation: A critical aim of this project is to use functional angiogenesis assays to assess vascular disruptive potential of a subset of ToxCast chemicals identified as pVDCs. Various *in vitro* platforms used to investigate vascular morphogenesis and differentiation include: transgenic zebrafish, whole embryo culture, human vascular endothelial cell culture, CAM assay,

rat aortic explant assay, and HTS of critical vascular biomarkers [(Kleinstreuer *et al.*, 2011; Knudsen and Kleinstreuer, 2012; McCollum *et al.*, 2011; Sarkanen *et al.*, 2010) and references therein]. The resulting data will be used to inform and optimize ToxCast assays for vascular disruption and assist in the interpretation of ToxCast HTS data, as well as to improve understanding of cross-species predictivity and refine modeling approaches.

We propose to determine whether putative vascular disrupting compounds impair angiogenesis using a cell-based angiogenesis assay, in collaboration with Eli Lilly, and an *in vivo* transgenic zebrafish assay.

Members of EPA's Integrated Systems Toxicology Division, National Center for Computational Toxicology, and the University of Houston are testing whether putative vascular disrupting compounds (pVDCs), identified in Kleinstreuer et al. 2011, disrupt angiogenesis in zebrafish (Figure 2). ToxCast pVDCs were identified by a suite of cell-based and biochemical assays that measure changes in gene and protein expression of key molecules involved in angiogenesis and map to AOPs for embryonic vascular disruption (Kleinstreuer et al. 2011). Currently, transgenic zebrafish are being used to detect vascular-specific toxicity *in vivo* by quantifying structural differences in vessel development (Figure 3) and testing whether vessels become patent. There is a need to confirm zebrafish results with another method that examines the effect of pVDCs on neovascularization. Thus, we propose to test a subset pVDCs and non-pVDCs in the angiogenesis assay to complement ongoing efforts in zebrafish by providing a secondary measurement of functional angiogenesis.

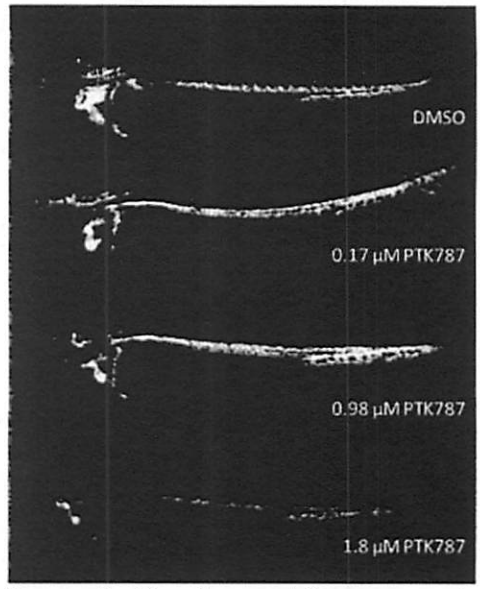

Figure 2: Developmental exposure to VEGFR2 inhibitor PTK787 impairs vessel development in transgenic

Tube formation, or the ability of endothelial cells to form three-dimensional structures, is one of a handful of cell-based assays that specifically tests for angiogenesis. The assay is commonly performed using a human endothelial cell line, and is routinely run by Eli Lilly as part of its suite of Phenotypic Drug Discovery assays. We anticipate that compounds that disrupt vessel development in zebrafish will also inhibit neovascularization in the angiogenesis assay.

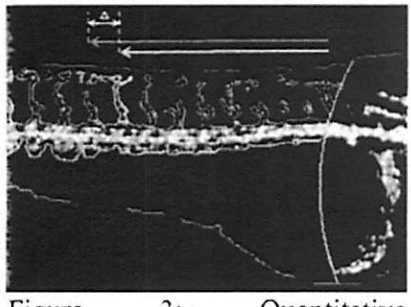

Figure 3: Quantitative measurements (vessel length or distance between vessels) can be extracted from images obtained on Cellomics high-content imaging system.

Approach: We propose to use the angiogenesis assay to assess ~36 chemicals in concentration response. The same chemicals and dose spacing used in the angiogenesis assay will be used in the zebrafish assays. To perform the study, we propose an MTA with Eli Lilly. The EPA will share with Lilly the zebrafish data

and other relevant assay results from ToxCast assays on the chemicals being tested. **Timeline:** We anticipate that it will take 6 months to perform the zebrafish study.
Resource needs: Cells, reagents and supplies to procure and run ~36 chemicals in Eli Lilly's angiogenesis assay in concentration response format.
Benefits: If successful, this will provide a measurement of functional angiogenesis, which we believe is critical to validate predictions derived from the ToxCast HTS data and support findings in the zebrafish model. It will also serve to help validate individual molecular targets that may be useful for anti-angiogenesis therapeutics.

CAS	Chemicals
96489-71-3	Pyridaben
28434-00-6	S-Bioallethrin
83657-24-3	Diniconazole
2312-35-8	Propargite
3380-34-5	Triclosan
314-40-9	Bromacil
114311-32-9	Imazamox
123312-89-0	Pymetrozine
81334-34-1	Imazapyr
300-76-5	Naled
137-26-8	Thiram
1897-45-6	Chlorothalonil
6317-18-6	Methylene bis(thiocyanate)
50-65-7	Niclosamide
111812-58-9	Fenpyroximate (Z,E)
35554-44-0	Imazalil
115-29-7	Endosulfan
41198-08-7	Profenofos
84852-15-3	4-Nonylphenol, branched
446-72-0	Genistein
57-83-0	Progesterone
123-31-9	Hydroquinone
1948-33-0	tert-Butylhydroquinone
97-77-8	Disulfiram
169590-42-5	Celecoxib
52-86-8	Haloperidol
101-20-2	Triclocarban
79902-63-9	Simvastatin
75330-75-5	Lovastatin
84371-65-3	Mifepristone
842-07-9	C.I. solvent yellow 14
50-41-9	Clomiphene citrate
532-27-4	2-Chloroacetophenone
1034-01-1	Octyl Gallate

5315-79-7	1-Hydroxypyrene
212141-51-0	PTK-787
	ToxCast Phase I
	ToxCast Phase II
	Positive Control

Kleinstreuer, N. C., Judson, R. S., Reif, D. M., Sipes, N. S., Singh, A. V., Chandler, K. J., Dewoskin, R., Dix, D. J., Kavlock, R. J. and Knudsen, T. B. (2011). Environmental impact on vascular development predicted by high-throughput screening. *Environ Health Perspect* **119**(11), 1596-603, 10.1289/ehp.1103412.

Sipes, N. S., Martin, M. T., Reif, D. M., Kleinstreuer, N. C., Judson, R. S., Singh, A. V., Chandler, K. J., Dix, D. J., Kavlock, R. J. and Knudsen, T. B. (2011). Predictive models of prenatal developmental toxicity from ToxCast high-throughput screening data. *Toxicol Sci* **124**(1), 109-27.

Knudsen, T. B. and Kleinstreuer, N. C. (2012). Disruption of embryonic vascular development in predictive toxicology. *Birth Defects Res C Embryo Today* **93**(4), 312-23, 10.1002/bdrc.20223.

Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrrano, J. A., Tietge, J. E. and Villeneuve, D. L. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* **29**(3), 730-41, 10.1002/etc.34.

McCollum, C. W., Ducharme, N. A., Bondesson, M. and Gustafsson, J. A. (2011). Developmental toxicity screening in zebrafish. *Birth Defects Res C Embryo Today* **93**(2), 67-114, 10.1002/bdrc.20210.

Sarkanen, J. R., Mannerstrom, M., Vuorenpaa, H., Uotila, J., Ylikomi, T. and Heinonen, T. (2010). Intra-Laboratory Pre-Validation of a Human Cell Based in vitro Angiogenesis Assay for Testing Angiogenesis Modulators. *Front Pharmacol* **1**, 147, 10.3389/fphar.2010.00147.