Science Advancement & Outreach
A DIVISION OF PETA

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Request for Information (RFI): Critical Challenges and Opportunities for Lymphatic Scientific, Clinical, and Disease Communities

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Science Advancement and Outreach

A division of People for the Ethical Treatment of Animals

We are writing on behalf of People for the Ethical Treatment of Animals—PETA entities have more than 9 million members and supporters globally—in response to this RFI from the National Commission on Lymphatic Diseases (NCLD). We provide a brief response on the topics numbered in the RFI, highlighting how novel methodologies can address current research gaps and propel the advancement of knowledge on lymphatic diseases. Recognizing the limitations of traditional animal models in studying the lymphatic system, we advocate for the adoption of cutting-edge human-based models and sophisticated computational approaches. These alternatives not only promise to enhance the accuracy and relevance of research findings but also align with ethical standards by reducing use of animals in experiments. **Our key recommendation is for the NCLD to pursue research using only human biology-based systems and not those using other species**.

We also invite NCLD to review PETA's strategy for advancing biomedical research in the U.S. through evidence-based practices and transitioning support to non-animal methods, applicable across various research domains. This plan, called Research Modernization Deal, can be accessed at https://www.peta.org/wp-content/uploads/2023/01/peta-research-modernization-deal.pdf, and we happy to meet to discuss any questions related to this response or the topics covered in the Research Modernization Deal.

For decades, lymphatic diseases have been overlooked due to low interest in studying the lymphatic system (LS) itself which has resulted in limited understanding about its related disorders.[1] Looking for opportunities to study the function of the LS and improve disease management, the National Heart, Lung, and Blood Institute (NHLBI) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), of the National Institutes of Health (NIH), hosted the virtual workshop "Yet to be Charted: Lymphatic System in Health and Disease" in September 2022,[2] which brought attention to the importance of the subject. Considering the broad nature of this RFI, we would like to provide a brief discussion focusing on two points: the future research landscape on the LS and the current limitations for clinical translation to humans.

Currently, rodents' tails are commonly used to study LS disorders. For these experiments, which predominately use mice, experimenters make an incision to the animal's tail to cut vessel flow and attempt to address the subsequent lymphatic pathogenesis.[3] However, these animal models have important drawbacks. First, no existing animal model preserves functional lymph collection vessel trunks, posing a major limitation in the relevance of the studies for investigating the idiopathy of lymphatic disorders and their evolution in humans, as these vessels remain intact after surgery.[4] As result, these experiments on animals focus on morphological changes in the epidermis that accompanies initial lymphangiogenic response,[3] a mechanism that is not relevant for humans. In addition, affected collecting vessels become enlarged, which is not observed in human biopsies,[5] and significant immune and physiology differences between humans and other animals make these experiments far from ideal to study lymphatic disorders.[6] For instance, a recent mouse tail model attempted to mimic pumping failure during lymphedema progression without disrupting the lymphatic collecting vessels. However, the model was irrelevant since the induced limb swelling resolved by itself in the mouse tail, even when untreated, due to compensatory non-lymphatic contractile mechanisms.

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[1] Jayathungage Don, T.D., Safaei, S., Maso Talou, G.D. *et al.* Computational fluid dynamic modeling of the lymphatic system: a review of existing models and future directions. Biomech Model Mechanobiol. 2024:23, 3–22.
[2] National Heart, Lung, and Blood Institute (NHLBI). News & Events: Yet to Be Charted: Lymphatic System in Health and Disease. Assessed on June 10, 2024. https://www.nhlbi.nih.gov/events/2022/yet-be-charted-lymphatic-system-health-and-disease.
[3] Rutkowski JM, Boardman KC, Swartz MA. Characterization of lymphangiogenesis in a model of adult skin regeneration. Am J Physiol Heart Circ Physiol. 2006;291(3):H1402-H1410.
[4] Weiler, M.J., Cribb, M.T., Nepiyushchikh, Z. et al. A novel mouse tail lymphedema model for observing lymphatic pump failure during lymphedema development. Sci Rep. 2019:9, 10405.
[5] F. Ogata, K. Fujiu, I. Koshima, R. Nagai, I. Manabe, Phenotypic modulation of smooth muscle cells in lymphoedema, British Journal of Dermatology. 2015:172(5), 1286–1293.
[6] Shou Y, Johnson SC, Quek YJ, Li X, Tay A. Integrative lymph node-mimicking models created with biomaterials and computational tools to study the immune system. *Mater Today Bio*. 2022;14:100269.

3D simulations on the impact of stiffness and leaflets (inserted on the vessel walls) on the function of lymphatic valves have been conducted using murine valves as a reference.[7,8,9] Similarly, numerous transgenic mouse models have been created in an attempt to reproduce human LS.[10,11] However, there are significant differences between human and mouse lymphatic vasculature, such as in geometry and microstructures.[12] Additionally, issues related to genetic manipulation, like off-target effects, along with the distinct physiology and immunology of mice, create substantial gaps in translating research findings to human-relevant results.[13,14] Computational models offer a valuable tool to simulate lymphatic functions in both health and disease,[1,4] generating extensive data that can be explored using *in vitro* models. Therefore, prioritizing human-based approaches in the context of lymphatic disorders is crucial for obtaining data translatable to humans.

Emerging bioengineered models now offer powerful alternatives to imaging and studying the lymphatic system, addressing the biomechanical limitations of experiments on animals.[4] Biomaterials such as hydrogels and microfluidic devices are excellent platforms for replicating human lymph node architecture and studying its physiology with continuous real-time measurements, challenges that are difficult to overcome with animal models.[4] Moreover, these biomaterials can be easily modified with specific biochemical and physical features, like incorporating extracellular matrix proteins into hydrogels,[15] to mimic the LS environment in a human-relevant manner.[4,5]

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[7] Bertram CD, Davis MJ. An Enhanced 3D Model of Intravascular Lymphatic Valves to Assess Leaflet Apposition and Transvalvular Differences in Wall Distensibility. Biology (Basel). 2023;12(3):379.
[8] Bertram CD. Modelling secondary lymphatic valves with a flexible vessel wall: how geometry and material properties combine to provide function. Biomech Model Mechanobiol. 2020;19(6):2081-2098.
[9] Li H, Mei Y, Maimon N, Padera TP, Baish JW, Munn LL. The effects of valve leaflet mechanics on lymphatic pumping assessed using numerical simulations. Sci Rep. 2019;9(1):10649.
[10] Redder E, Kirschnick N, Bobe S, Hägerling R, Hansmeier NR, Kiefer F. Vegfr3-tdTomato, a reporter mouse for microscopic visualization of lymphatic vessel by multiple modalities. PLoS One. 2021;16(9):e0249256.
[11] Doh SJ, Yamakawa M, Santosa SM, Montana M, Guo K, Sauer JR, Curran N, Han KY, Yu C, Ema M, Rosenblatt MI, Chang JH, Azar DT. Fluorescent reporter transgenic mice for in vivo live imaging of angiogenesis and lymphangiogenesis. Angiogenesis. 2018:21(4):677-698.
[12] Xiang M, Grosso RA, Takeda A, Pan J, Bekkhus T, Brulois K, Dermadi D, Nordling S, Vanlandewijck M, Jalkanen S, Ulvmar MH, Butcher EC. A Single-Cell Transcriptional Roadmap of the Mouse and Human Lymph Node Lymphatic Vasculature. Front Cardiovasc Med. 2020:30;7:52.
[13] Lampreht Tratar U, Horvat S, Cemazar M. Transgenic Mouse Models in Cancer Research. Front Oncol. 2018;8:268.
[14] Li Z, Zheng W, Wang H, et al. Application of Animal Models in Cancer Research: Recent Progress and Future Prospects. Cancer Manag Res. 2021;13:2455-2475.
[15] Kim J, Wu B, Niedzielski SM, Hill MT, Coleman RM, Ono A, Shikanov A. 2015. Characterizing natural hydrogel for reconstruction of three-dimensional lymphoid stromal network to model T-cell interactions. J Biomed Mater Res Part A 2015:103A: 2701–2710.

Questions remain about the relationship between lymphatic pumping failure and the LS due to a lack of appropriate experimental models.[3] Innovative 3D models using bioprinted[16] or cultivated[17,18] human lymphatic endothelial cells are helping to elucidate lymphatic function in its microenvironment, which cannot be recreated with experiments on animals. This presents an opportunity for NCLD to support these patient-derived models to investigate lymphatic function and to develop contractile *in vitro* models in a human-relevant context.[19]

Many examples of human lymph nodes-on-chip (LNoC), designed with different materials, are detailed in a 2021 review by Shanti, *et al*. in *Frontiers in Pharmacology*.[20] Recently, a new LNoC supported the formation of lymph nodes with active germinal centers from primary human blood cells.[21] This human-derived 3D model and others[22] enable the study of immune response to immunization and the recreation of tissue structure as found *in vivo;* and highlight the potential of these non-animal methods for biomedical research and high-throughput drug screening applications.[4] Additionally, there is promise in regenerative medicine for transferring functional immune cells, such as in lymphadenectomy and lymphodepletion.[23] To make significant advancements in understanding and treating lymphatic diseases, NCLD must provide the necessary financial and infrastructural support to these more reliable, non-animal models. For more detailed recommendations on building more efficient and innovative biomedical research, please refer to the Research Modernization Deal: https://www.peta.org/wp-content/uploads/2023/01/peta-research-modernization-deal.pdf.

Early diagnosis of lymphatic disorders is challenging and there are techniques that can accelerate screening process and patients' selection for optimal intervention strategies.[4] For instance, NCLD can develop programs that include the collection of genetic data from patients to determine biological and

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[16] Cao X, Ashfaq R, Cheng F, et al. A Tumor-on-a-Chip System with Bioprinted Blood and Lymphatic Vessel Pair. Adv Funct Mater. 2019;29(31):1807173.
[17] Gong MM, Lugo-Cintron KM, White BR, Kerr SC, Harari PM, Beebe DJ. Human organotypic lymphatic vessel model elucidates microenvironment-dependent signaling and barrier function. Biomaterials. 2019;214:119225.
[18] Rogic A, Auger F, Skobe M. Isolation of Human Skin Lymphatic Endothelial Cells and 3D Reconstruction of the Lymphatic Vasculature In Vitro. Methods Mol Biol. 2018;1846:279-290.
[19] Henderson AR, Choi H, Lee E. Blood and Lymphatic Vasculatures On-Chip Platforms and Their Applications for Organ-Specific In Vitro Modeling. Micromachines (Basel). 2020;11(2):147.
[20] Shanti A, Hallfors N, Petroianu GA, Planelles L, Stefanini C. Lymph Nodes-On-Chip: Promising Immune Platforms for Pharmacological and Toxicological Applications. Front Pharmacol. 2021;12:711307
[21] G. Goyal, B. Bausk, P. Prabhala, L. Xie, D. Curran, J. Long, L. Cohen, O. Levy, R. Prantil-Baun, D.R. Walt, D.E. Ingber, Lymph node follicle formation and vaccination responses reconstituted in vitro in a human Organ Chip. BioRxiv (2019) 806505.
[22] Votanopoulos, K.I., Forsythe, S., Sivakumar, H. et al. Model of Patient-Specific Immune-Enhanced Organoids for Immunotherapy Screening: Feasibility Study. Ann Surg Oncol 2020:27, 1956–1967
[23] J. Najibi, J. Mooney. Cell and tissue engineering in lymph nodes for cancer immunotherapy. Advanced Drug Delivery Reviews. 2020:161–162.

environmental influence, such as obesity or cancer,[24,25,26] on lymphatic disorder prevalence. In parallel, to advance understanding of the LS, it is crucial to educate care providers and researchers. One way to support progress is by training the medical and scientific community in modern technologies for lymphatic visualization that allow the investigation of questions unmet by traditional experiments on animals, including anatomic variations and abnormalities of the LS.[1,7,27]

Much of our understanding of the lymphatic system's anatomy has been derived from cadaver visualization.[28] Advancements in lymphatic research have been impeded by the challenges of visualizing the live LS, which cannot be achieved with a single injection of contrast agents.[29] For example, indocyanine green (ICG) lymphography, a commonly used imaging method, employs near-infrared cameras to detect injected ICG but has several limitations. Over the past decade, new lymphatic mapping techniques, such as computed tomography lymphangiography and dynamic contrast-enhanced magnetic resonance lymphangiography,[30] have been developed to offer enhanced resolution and sensitivity for a significant portion of the LS. NCLD could foster partnerships with companies and hospitals to increase human sampling, thereby accelerating the development and implementation of these advanced imaging technologies.

The pathophysiology of LS diseases also remains elusive or poorly understood as lymphatic variants and comorbidities seem to be implicated in lymphatic disorders.[31] Secondary lymphedema, a swelling of limbs affecting over 130 million individuals,[3] is prevalent in 43% to 94% breast cancer patients who underwent radiotherapy at five years.[32] Although management is primarily physiotherapy and decongestive lymphatic therapy,[4] there is a need to improve early diagnosis and technologies such high-definition ultrasonography and bioimpedance spectroscopy, which are in high demand by the clinical community.[14]

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[24] Sleigh BC, Manna B. Lymphedema. StatPearls. Treasure Island (FL): StatPearls Publishing. Updated 2023 Apr 19. 2024 Jan-. Assessed June 17, 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537239/

[25] J Green. Lymphedema Risk Factors: Who's at Risk for Lymphedema. Tactile Medical. Published March 12, 2024. Assessed June 17,2024. Available at: https://tactilemedical.com/resource-hub/lymphedema/lymphedema-risk-factors-whos-at-risk-for-lymphedema/#4

[26] Montagna G, Zhang J, Sevilimedu V, et al. Risk Factors and Racial and Ethnic Disparities in Patients With Breast Cancer-Related Lymphedema. JAMA Oncol. 2022;8(8):1195-1200. doi:10.1001/jamaoncol.2022.1628

[27] Pieper CC, Feisst A, Schild HH. Contrast-enhanced Interstitial Transpedal MR Lymphangiography for Thoracic Chylous Effusions. Radiology. 2020;295(2):458-466.

[28] Singhal D, Börner K, Chaikof EL, et al. Mapping the lymphatic system across body scales and expertise domains: A report from the 2021 National Heart, Lung, and Blood Institute workshop at the Boston Lymphatic Symposium. Front Physiol. 2023;14:1099403.

[29] Munn LL, Padera TP. Imaging the lymphatic system. Microvasc Res. 2014;96:55-63.

[30] Ramirez-Suarez, K.I., Tierradentro-Garcia, L.O., Smith, C.L. et al. Dynamic contrast-enhanced magnetic resonance lymphangiography. Pediatr Radiol. 2022:52, 285–294.

[31] Mehrara BJ, Radtke AJ, Randolph GJ, et al. The emerging importance of lymphatics in health and disease: an NIH workshop report. J Clin Invest. 2023;133(17):e171582.

[32] Rockson, S.G., Keeley, V., Kilbreath, S. et al. Cancer-associated secondary lymphoedema. Nat Rev Dis Primers. 2019:5, 22.

As mentioned by Mehrara and colleagues in their report, "The emerging importance of lymphatics in health and disease,"[13] improving patient care necessitates the creation of human-based studies, including epidemiological and longitudinal research, as well as prospective trials to characterize and predict risks. Moreover, identifying lymphatic biomarkers and molecular characterization of lymphatic muscle cells through the assessment of clinical samples from biobanks could significantly advance the development of future therapies.[13] NCLD can address this need by establishing multicentric studies in partnership with institutions specializing in LS and specific patient groups, such as those with breast cancer.

Another promising area is vascularized lymph node transplantation (VLNT) for lymphedema treatment. For VLNT, lymph nodes from a healthy donor are transplanted to the affected limb of the patient with an arterial and venous anastomosis.[33] Longitudinal studies reported great clinical benefit in patients two years after VLNT, with improved limb circumference and compression, including breast cancer patients, with low complication risk.[15,34,35,36] Physicians have reinforced the need for more patient-based studies to fully characterize VLNT for lymphedema and to improve outcome. Therefore, we encourage NCLD to prioritize funding to longitudinal studies that can help the understanding of LD and provide effective interventions for human patients instead of continuing to support experiments on animals that fail to provide relevance to human physiology.[4]

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[33] Brown S, Mehrara BJ, Coriddi M, McGrath L, Cavalli M, Dayan JH. A Prospective Study on the Safety and Efficacy of Vascularized Lymph Node Transplant. Ann Surg. 2022;276(4):635-653.
[34] Schaverien MV, Asaad M, Selber JC, et al. Outcomes of Vascularized Lymph Node Transplantation for Treatment of Lymphedema. J Am Coll Surg. 2021;232(6):982-994.
[35] Chang EI, Ibrahim A, Liu J, et al. Optimizing Quality of Life for Patients with Breast Cancer-Related Lymphedema: A Prospective Study Combining DIEP Flap Breast Reconstruction and Lymphedema Surgery. Plast Reconstr Surg. 2020;145(4):676e-685e.
[36] Coroneos CJ, Asaad M, Wong FC, et al. Outcomes and technical modifications of vascularized lymph node transplantation from the lateral thoracic region for treatment of lymphedema. J Surg Oncol. 2022;125(4):603-614.