Lymphatic Education
& Research Network

GAPS IN THE FIELD OF LYMPHATIC RESEARCH

LD and LE Research Gaps Submitted to CDMRP for FY2023 Funding Consideration

In FY2023, Lymphatic Disease and Lymphedema Research are congressionally mandated "Topic Areas" fundable for research under the **Congressionally Directed Medical Research Program (CDMRP)**. The CDMRP is a funding organization, located within the Department of Defense, that strives to fund innovative and impactful biomedical research driven by the needs of our stakeholders—service members, veterans, beneficiaries, the American public, and Congress.

The **Peer Review Medical Research Program (PRMRP)** has been provided the following prioritized bulleted list of research gaps, emphasizing the most urgent need(s) in patient care for Lymphatic Diseases.

LE&RN would like to acknowledge our research community and the National Institute of Health (NIH) who hosted the *Yet to Be Charted: Lymphatic System in Health and Disease* research workshop, Sept. 19–20, 2022. We especially thank the presenters who—by identifying knowledge gaps and opportunities in lymphatic disease research—made this list possible as we report a summary of their findings here.

CLINICAL GAPS

1. Early Diagnosis

One of the most frustrating aspects of caring for patients living with lymphatic disease is the relative lack of highly sensitive and specific non-invasive tests and blood biomarkers. Without these essential diagnostic tools, a delay in diagnosis is inevitable and often results in increased morbidity and mortality.

Imaging:

- *An urgent need in novel non-invasive imaging techniques and devices.*
- A need for further investigation of proposed techniques:
  - Fat-weighted whole-body MRI (ex. Lipedema–fat to water ratio is greater than obesity)
  - Water-weighted MRI of soft tissue
  - Standardized sodium 23NA-MRI
  - MR Lymphangiogram techniques
  - Non-tracer MRL sensitive to edema and vascular morphology
  - While we currently are using ICG imaging—we need to develop it to yield quantitative data that will permit comparative analysis.
  - Other—PET? Functional MRI?

There is a clinical need for development of *noninvasive imaging of lymphatic physiology* to aid diagnosis and understanding of all lymphatic diseases, including lymphedema & lipedema. (Example: MRI Tools needed: noninvasive MRL, anatomical and sodium MRI, spectrometry MRI).

Biomarkers:

- Development of non-invasive assessment for genotyping of somatic mutations that are safer than biopsy (ex. circulating free DNA).
- Novel biomarkers utilizing bodily fluids (urine, cyst fluid, pleural effusions, edematous fluid, saliva, and blood).

Omics:

Transcriptomics, proteomics and metabolomics, and microbiome studies:

- Define transcriptional machinery of lymphangiogenic pathways.
- Characterizing the role of the lymphatic vasculature in immune activation (outside lymphoid organs).
- Developing therapeutic lymphangiogenesis concepts.
- In solid organ transplants, would competent lymphatics enhance graft health?
- In breast cancer patients, can preemptive and targeted therapy safely accelerate lymphatic repair?
- Single Cell RNA techniques and atlas mapping
- How does the microbiome affect lymphatic health?

Standardized Definitions of Disease:

- Need objective documentation of appropriate standards for the diagnosis of lymphedema, lipedema, and other chronic edematous conditions.
- Need the study of lymphatic medicine included as a discipline taught in medical school and included as an integral part of the cardiovascular system.
- We need to better characterize the sex-specific patterns of obesity and regional adipose definition in order to understand when the pathology of lipedema is present.
- Develop and measure core outcome measures for lymphedema/lymphatic disease therapists to optimize clinical care and bring consistency to lymphedema/lymphatic disease research outcome goals.

2. Lymphatic Specific Pharmacotherapies and Targeted Therapies

- We lack medications that improve lymphatic function without causing significant off-target toxic effects.
- We lack drug mechanism studies.
- How do we evaluate drug resistance (pathway investigation)?
- Inhibition of lymphatic signaling in tumor metastasis.
- Novel methods of drug delivery (viral vectors, CART-T?)
- Is there a role for pharmacology in lymphedema prevention?
- Is there a role for pharmacology in lipedema prevention or treatment?
- Does leukotriene biology offer opportunities for related lymphatic diseases like vascular malformation, lymphagiectasia, lipedema and others?
- Causes and interventions for complications of lymphatic disease and lymphedema (ex. lymphatic pain, disfiguration, fibrosis, and coagulopathy)

3. Therapeutic Devices, Surgical or Interventional Radiology Treatments of Lymphedema or other Lymphatic Diseases

- How can the strategy of surgical prevention of lymphedema in cancer operations or treatment of other lymphatic diseases be further refined with regard to technique?
- Longer-term outcomes need to be evaluated for lymph node transfer and lymphaticovenous anastomosis for lymphedema.
- Further research will help determine for which patients vascularized lymph node transfer and/or lymphaticovenous anastomosis is effective to aid patient selection.
- Large-scale prospective clinical trials are needed to determine optimal treatment algorithms. (Some do well, but those with an inflammatory component to lymphedema do not do as well.)
- Mechanisms of immunomodulation following vascularized lymph node transplant need to be elucidated to aid development of pharmacological therapies.
- Need for large scale prospective trials analyzing outcomes of lymphedema surgery with a goal of defining optimal surgical algorithms and analyzing these outcomes long-term. Studies like this are needed for insurance coverage.
- Understanding post-surgical lymphedema prevention and long-term surveillance for current breast cancer surgical reconstruction procedures.
- How to improve and tailor Phase 2 Complete Decongestive Therapy self-management activities for the survivor living with lymphedema.
- Role of complete decongestive therapy in rehabilitation pre- and post-surgical treatment.
- Examine the adherence and outcomes of people living with lymphedema to use of pneumatic compression devices across the lifespan.
- Novel compression device development and comparison studies with current approved devices.
- Interventions to manage lymphedema in advanced cancer and innovation in palliative care.
- Need for further development of novel techniques in magnetic resonance lymphangiography.
- Need for novel interventional radiology preventative and therapeutic approaches to lymphatic disease.
- Need for interventional radiology drug delivery options.

4. Epidemiologic and Longitudinal Data

- Need for epidemiologic studies to realistically determine the incidence and prevalence of lymphatic disease in the US (primary, secondary lymphedema, lipedema, vascular malformations and other rare lymphatic disorders).
- We lack natural history studies for lymphatic diseases and require them for FDA clinical trials.
- Broader and more comprehensive and collaborative registries are needed.

5. Access to Care and Global Health

- Rural and global health disparities in people living with lymphedema.
- Impact of climate change on chronic edema/lymphedema.
- Remote education/telehealth interventions to support those living remotely and rural people living with lymphedema.

6. Standards for Outcomes Measures

- Development of validated patient reported outcomes in lymphatic disease.
- Development of validated clinical outcomes (i.e., radiologic measures).

7. Patient Advocates Emphasized These Gaps (Data Collected from the NIH 2022 *Yet to Be Charted: Lymphatics in Health and Disease* Research Workshop)

- Improved awareness and specialized training for medical staff to recognize, diagnose and treat lymphatic diseases.
- Improved imaging of lymphatic disorders.
- Non-invasive diagnostics and emphasis placed on establishing lymphatic medicine as a discipline.
- Need for more Centers of Excellence and need for more specialists.
- A need for primary care givers to be more educated in lymphatic disorders.
- Need for creative state-of-the-art surgical approaches.
- Need for methods of "transition of care" from pediatric to adult vascular anomaly patients and specialty centers for pediatric and adult vascular anomaly patients.
- Need a method to not forget but instead include psychosocial counseling for patients with lymphatic diseases that have been historically neglected.
- Identify the barriers to the routine referral of at-risk people for lymphatic disease and/or lymphedema assessment, preventative exercises, self-management education, and treatment as needed across the lifespan.

BASIC & TRANSLATIONAL SCIENCE GAPS

1. The Developmental Origin of the Lymphatic System

Including often neglected lymphatic muscle cells (where do lymphatic muscle cells originate in development?), contractile protein and ion channel expressions of lymphatics, and the regional differences.

2. Quantification of Lymphatic Function

We lack an understanding of the pressures and flows in healthy and dysfunctional lymphatic networks.

- Mechanisms that maintain vessel homeostasis and role in optimizing function.
- We lack functional studies of human vessels, especially of healthy vessels.
- We lack studies to better understand the extent to which lymphatic contractile and valve dysfunction result from, or contribute to, other pathologies.
- Because it is technically difficult to study human lymphatic smooth muscle cells, this has resulted in relying on the study of lymphatic endothelial cells. We have a paucity of research on the diversity and function of lymphatic smooth muscle cells. This includes the developmental origin of lymphatic muscle cells, contractile protein and ion channel expressions of lymphatics, and the regional differences.
- Further evaluation required to understand the consequences of having or not having a normal functioning thoracic duct.
- Do primary lymphedema causing genetic mutations affect lymphatic vessel contractility and permeability?

3. Evaluation of Lymphatic Anatomy

- Need to map the entire human lymphatic system in health and in disease.
- What are the main lymphatic anatomy variants of the upper extremity, lower extremities, and the body in general?
- How does the presence or absence of any anatomic variant potentially contribute to the incidence of lymphedema or other lymphatic diseases?

4. Need for Accurate and Widely Available Animal Models

A major gap and need in studying lymphatics is the availability of animal models that recapitulate the diverse biology and heterogeneity of human lymphatics (especially organ specific lymphatics in health and disease). For example: aspects of mouse lymphatic physiology do not apply to humans; i.e., visceral and thoracic collecting lymphatics do not pump, and mice are not exposed to chronic gravitational loads. No animal models exist for the human condition of lipedema.

5. Lymphatic Endothelial Cell (LEC) Specification

- We need to Identify genes controlling the earliest steps of lymphatic endothelial cell specification.
- Development of in vitro systems to derive lymphatic endothelial cells from human embryonic stem cells.
- Determine the plasticity of LEC phenotype and its regulation by metabolic factors.
- We need to identify relevant molecules and cells with which LECs communicate *in vivo*.

- How do mutant endothelial cells affect normal endothelial cells.
- How do complex lymphatic anomaly-causing genetic mutations affect different lymphatic EC populations and how do mutant LECs affect normal LECs?

6. The Role of the Lymphatic System in Different Organs of the Human Body in Health and in Disease

How and why do lymphatics of certain organs become dysfunctional, causing disease?

Examples of organ specific gaps in lymphatic knowledge:

- Lymphatics of the eye –> gaps in understanding pathophysiology of glaucoma, retinal and optic nerve diseases, and corneal transplant rejection .
- Lymphatics of the liver (a key organ of lymph production) -> gaps in understanding the molecular mechanisms of lymphangiogenesis, the role of the lymphatic system in different etiologies of liver disease, the modulation of hepatic lymphatic system as strategy for treating liver disease and its complications.
- Lymphatics of the heart -> gaps in understanding lymphatic signaling vs. lymphatic drainage function during cardiac repair following MI, does lymphatic signaling affect other cell types in the heart during development of cardiac disease, understanding the origins of injury induced lymphatic vessels, need to identify the novel players regulating lymphatic sprouting during embryonic development.
- Lymphatics of the GI tract -> gaps in understanding whether lymphatics of the GI are a conserved feature of epithelial cell niches (local microenvironment), does lymphatic intestinal stem cell communication respond dynamically to infection, inflammation, changes in nutrient availability, how do stem cells and crypt-based lymphatics regulate access of immune cells to the intestinal stem cell niche, how might stem cells regulate their lymphatic niche? How lymphatic endothelial cells sense and respond to tissue alterations, microbiome, and immune cues? What are the homeostatic lymphatic endothelial cells role in the intestine and its implication in disease?
- Lymphatics of kidneys -> there is evidence that promotion of lymphangiogenesis is beneficial in several kidney diseases. But why is that? Studies demonstrate that there is great heterogeneity of lymphatic endothelial cells across microvascular beds, vascular cell type, developmental stages, health vs. disease states. But why is that?

We need to better understand the lymphatic substrates (wall problem, valve problem, or pumping problem) that results in lymphatic failure with organ dysfunction (when lymph becomes dysfunctional how does it affect the organ).

We need to better understand the contribution of lymphatic dysfunction to end organ dysfunction.

We need to develop better treatment options for patients with lymphatic failure due to organ dysfunction.

7. How are Lymphatics Involved with Obesity, Aging, and Chronic Inflammation?

We need a better understanding of the lymphatic system and lymphatic valves in obesity, lipedema, and lymphedema.

8. Bench to Bedside Technologies

- Need to understand the gaps in pathological processes that have been identified such as inflammation and lymphangiogenesis.
- Need to identify causative mutations in novel genes underpinning primary lymphedema and complex lymphatic and vascular anomalies in humans.

Basic Science Research: Gaps and Needs for Rare Lymphatic Diseases

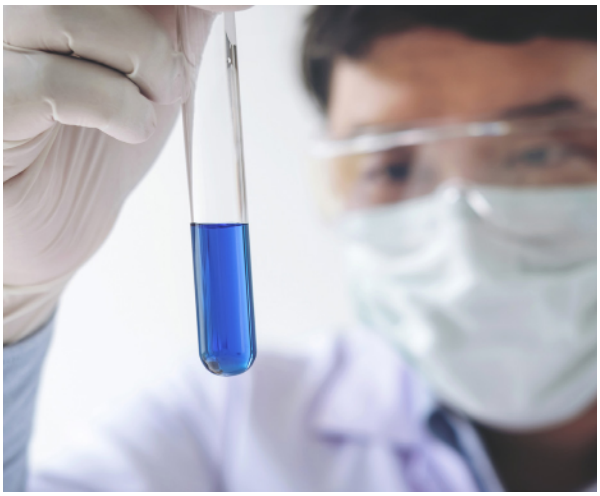
- How is the microenvironment altered in complex lymphatic anomaly (CLA) tissues and does it contribute to disease progression?
- What is the full complement of CLA-causing genetic mutations? Is it more than "one hit" that causes mutations?
- What makes the responsible somatic mutations for CLAs different from cancer causing mutations?
- What is the best method to genotype patients? Can we use cell-free DNA or circulating biomarkers to diagnose CLAs and monitor response to treatments?
- Are there other circulating diagnostic biomarkers?
- How do CLA-causing genetic mutations affect different lymphatic endothelial cell (LEC) populations and do mutant LECs affect normal LECs?
- Do CLA-causing genetic mutations affect lymphatic vessel contractility and permeability?
- What other genes, factors & pathways contribute to the pathogenesis of vascular and lymphatic anomalies.
- How do mutant endothelial cells affect normal endothelial cells.
- Why do lymphatics invade bone? Is it just by chance?

- What causes coagulopathy (trapping of platelets and clotting factors) in some patients presenting with complex lymphatic anomalies?
- What causes the lymphatic endothelial cells to become spindle shaped? Do these abnormal cells affect surrounding cells? How?
- Is crosstalk between lymphatic endothelial cells (LECs) and osteoclasts important for the progression of CLAs?
- We need cell lines that will allow in vitro work and will allow in vivo xenografts or allografts.
- We need genetically engineered animal models
- We need a repository of biospecimens (blood, body fluids, tissue).

Clinical Science Research: Gaps and Needs for Rare Lymphatic Diseases

- There are big gaps in the collaborative collection of data and biospecimens for rare lymphatic and vascular anomalies.
- We need Natural History Studies.
- We need broader, more comprehensive, and collaborative registries.
- Gaps in early diagnosis and therefore with delayed diagnosis comes morbidity and mortality.
- We need more non-invasive diagnostic tests and biomarkers for lymphatic and vascular anomalies.
- Drug mechanism studies: how does sirolimus or any drug used currently for CLAs work (ex. Trametinib, Apelesib), how do we assess PK response? How do we evaluate drug resistance (pathway investigation) with all of these drugs?
- Do we need dual therapy? Working on both the PIK3 and RAS pathways?
- Better evaluation of response (radiologically). Standards for outcome measures.
- Development of validated patient reported outcomes and other clinical outcomes.
- Other assessment for genotype that are safer than biopsy (circulating free DNA).
- Need federal legislation for clinical trials and genomic testing.
- Need more pilot studies for novel therapies.
- Need orphan drug designations of medications.
- Need PK analysis for pediatric dosing.
- Need novel methods for drug delivery directly into affected tissue and cells to avoid the toxic side effect profile of existing and new drugs. (Where good animal models are urgently needed.)
- Need to be able to target mutant endothelial cells and reduce toxic side effects of drugs used to treat.
- Is trametinib a better treatment for RAS pathway driven CLAs than sirolimus? What other MEK-inhibitors may be better with less side effects?
- How do mutations in genes that cause CLAs affect initial lymphatics and collecting lymphatics?
- Are there therapies besides obvious targeted therapies that would be effective at treating CLAs?
- Can the disease be monitored by imaging (e.g., FDG-PET, fMRI, other novel techniques)?
- Why do mutations in PIK3CA cause different phenotypes?





Lymphatic Education & Research Network

154 West 14th Street, 2nd Floor • New York, NY 10011
Phone: (516) 625-9675 • Fax: (516) 625-9410
E-mail: lern@LymphaticNetwork.org

www.LymphaticNetwork.org