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PRINCIPAL INVESTIGATOR: Dr. Michael Dellinger, Jr. PhD

CONTRACTING ORGANIZATION: University of Texas Southwestern Medical Center

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Sporadic lymphatic malformations (LMs) are chronic, progressive and debilitating diseases caused by errors in the development of the lymphatic vasculature. These diseases include generalized lymphatic anomaly, kaposiform lymphangiomatosis, Gorham-Stout disease, and central conducting lymphatic anomaly. LMs can result in life-threatening complications and the 5-year survival rate is as low as 51% for certain LMs. Several different treatments are used to manage the symptoms of LM patients and in recent years sirolimus (FDA-approved mTOR inhibitor) has become the standard of care for patients. However, not all patients respond to sirolimus. Therefore, there is an urgent need for new pharmacotherapies for sporadic LMs. We and others have recently identified somatic activating mutations in KRAS in LM patients. KRAS is a small G-protein that activates the RAF/MEK/ERK signaling pathway and the mechanism by which it causes lymphatic dysplasia is poorly understood. We have found that mice that express an active form of KRAS in their lymphatics (iLECKras mice) develop fewer lymphatic valves than control mice. We have also found that hyperactive KRAS signaling in lymphatics affects the localization of VE-Cadherin, a protein that is required for fluid shear stress signaling and lymphatic valve development and maintenance. The objectives of this application are to determine the mechanisms by which excessive KRAS signaling impairs lymphatic function and to test the ability of an FDA-approved MEK inhibitor to reverse lymphatic defects in our clinically relevant mouse model. Hyperactive KRAS signaling causes the disintegration of lymphatic valves by inhibiting VEcadherin signaling and that this can be reversed with trametinib.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. INTRODUCTION:

Lymphatic malformations (LMs) are life-threatening diseases caused by abnormal development of lymphatic vessels. Current treatments for LMs include surgery, sclerotherapy, and sirolimus. However, these treatments are inadequate for many patients. Therefore, there is an urgent need for new therapies for LMs. We and others have recently identified genetic mutations in *KRAS* in LM patients. The mechanisms by which *KRAS* mutations cause LMs are poorly understood. We have found that mice that express a mutant form of *KRAS* in their lymphatic vessels develop fewer lymphatic valves than control mice. Lymphatic valve defects cause retrograde lymph flow and deadly complications in patients. In this project, we are investigating the mechanisms by which KRAS affects the maintenance of lymphatic valves (**Aim 1**). We are also testing the effect of an FDA-approved MEK inhibitor (trametinib) on aberrant lymphatic development in our clinically relevant LM mouse model (**Aim 2**). The long-term impact of our work is that it could lead to the repurposing of FDA-approved MEK inhibitors for a spectrum of LMs and enable a precision medicine approach for treating these life-threatening diseases. Filling this unmet medical need could improve patient outcomes and help alleviate the stress experienced by civilians, military service members, and veterans raising a child with a LM.

2. KEYWORDS:

KRAS, lymphangiogenesis, lymphatic valve, lymphatic malformation, vascular malformation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The project contains two aims as stated in the SOW:

- Aim 1: Identify the molecular mechanisms by which excessive KRAS signaling in LECs causes the disintegration of lymphatic valves.

- Aim 2: Test the ability of an FDA-approved inhibitor to prevent and/or reverse phenotypes caused by excessive KRAS signaling in LECs.

What was accomplished under these goals?

Aim 1: Identify the molecular mechanisms by which excessive KRAS signaling in LECs causes the disintegration of lymphatic valves.

Time course of lymphatic valve disintegration in Flt4CreER^{T2};Kras^{G12D};Prox1GFP mice.
To determine the timing of when the lymphatic valves disintegrate or are lost upon expressing the KRAS-G12D protein, we first assessed the *Prox1CreER^{T2};Kras^{G12D};Prox1GFP* mice that we proposed to study. We found that the transgenic *Prox1CreER^{T2}* strain resulted in early postnatal lethality, preventing us from evaluating valve development over time. To overcome this limitation, we mated the new and lymphatic-restricted *Flt4CreER^{T2}* strain with the *Kras^{G12D}* allele and characterized the resulting *Flt4CreER^{T2};Kras^{G12D};Prox1GFP* mice and *Flt4CreER^{T2};Prox1GFP* controls (**Figure 1**). Lymphatic valves were counted and normalized to total vessel length

(valves/mm), and lymphatic vessel diameter was also measured. Overall, we found a ~90% loss of lymphatic valves in every tissue examined and lymphatic vessel diameter was significantly enlarged

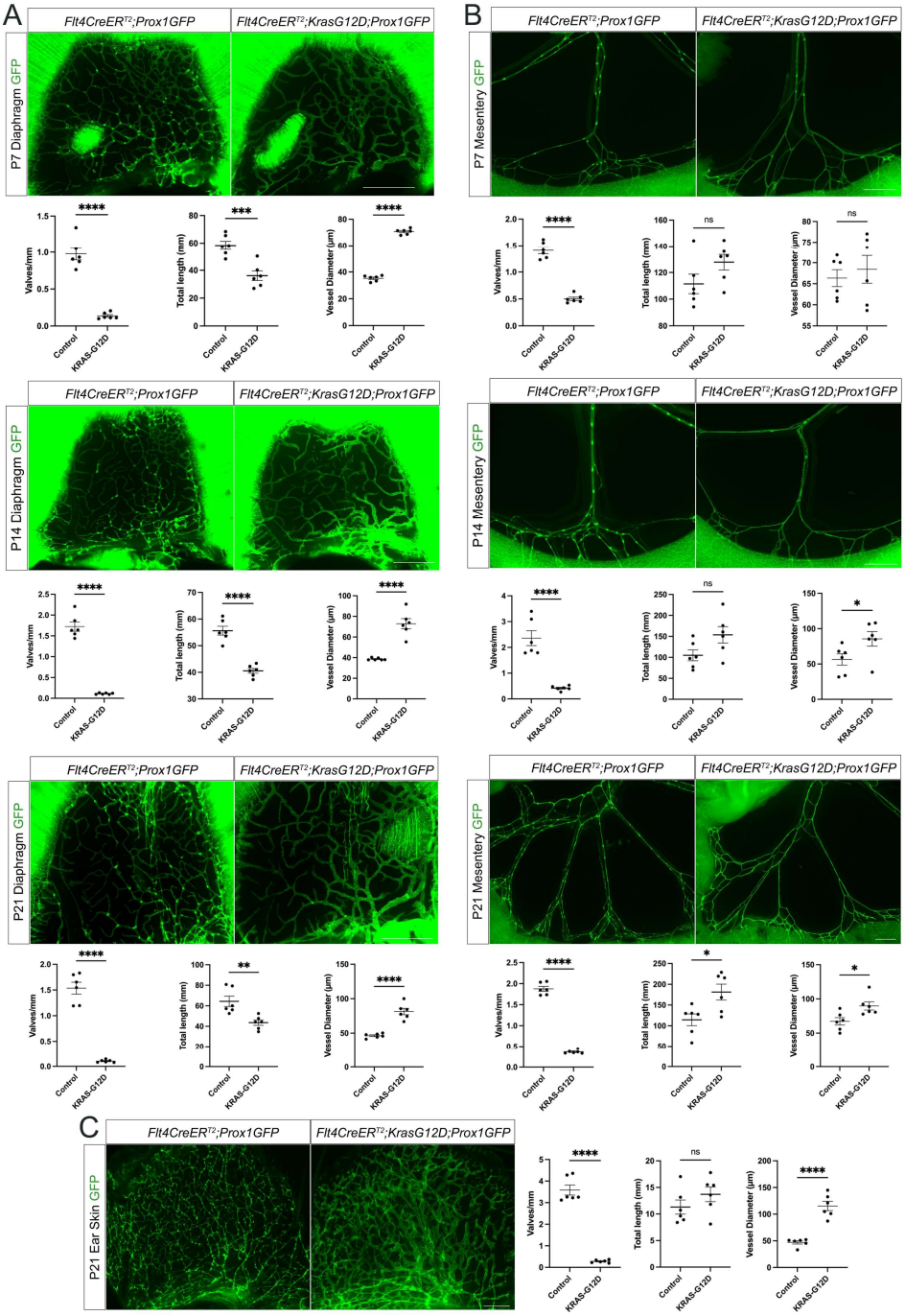

Figure 1. Valve quantification for diaphragm, mesentery, and ear skin for *Flt4CreER^{T2};Prox1GFP* (control) and *Flt4CreER^{T2};Kras^{G12D};Prox1GFP* mice. A. The number of valves/mm and vessel diameters were quantified in diaphragm tissues from control and KRAS-G12D-expressing mice at postnatal days P7, P14, and P21. **B.** The number of valves/mm and vessel diameters were quantified for the mesenteric lymphatic vessels at the same postnatal days, as indicated. **C.** The number of valves/mm and vessel diameters were quantified for the ear skin lymphatic vessels at the same postnatal days, as indicated.

in the diaphragm and ear skin, but not in the mesentery, when compared to controls.

Contribution of shear stress signaling to the changes in gene expression induced by KRAS-G12D. In our preliminary data for this grant application, we found that cultured LECs expressing the KRAS-G12D protein markedly downregulated the endothelial junction protein, VE-cadherin. This has been shown for other RAS/MAPK mutations in the lymphatic vasculature (e.g. ARAF-S214P) as well as blood endothelial cells carrying a KRAS mutation. Because we have established that VE-cadherin controls mechanotransduction signaling in response to oscillatory shear stress (OSS) in LECs, we tested the hypothesis that LECs expressing KRAS-G12D downregulate lymphatic valve genes due to a loss of mechanotransduction signaling. We cultured LECs infected with a control vector (mCherry) or KRAS-G12D in the absence or presence of OSS for 48 hours prior to performing qRT-PCR for lymphatic valve genes (**Figure 2**). First, we found that many of the shear-responsive genes were increased in control LECs exposed to OSS. Second, we observed that the genes critical for valve development were massively downregulated. Finally, we show that the downregulation of valve genes occurs regardless of whether or not shear stress is present. Although flow-responsive genes are downregulated, this occurs even in static conditions when VE-cadherin is not needed for active signaling. Therefore, we conclude that shear stress is not necessary for the loss of valves in the KRAS-G12D expression LECs *in vivo*.

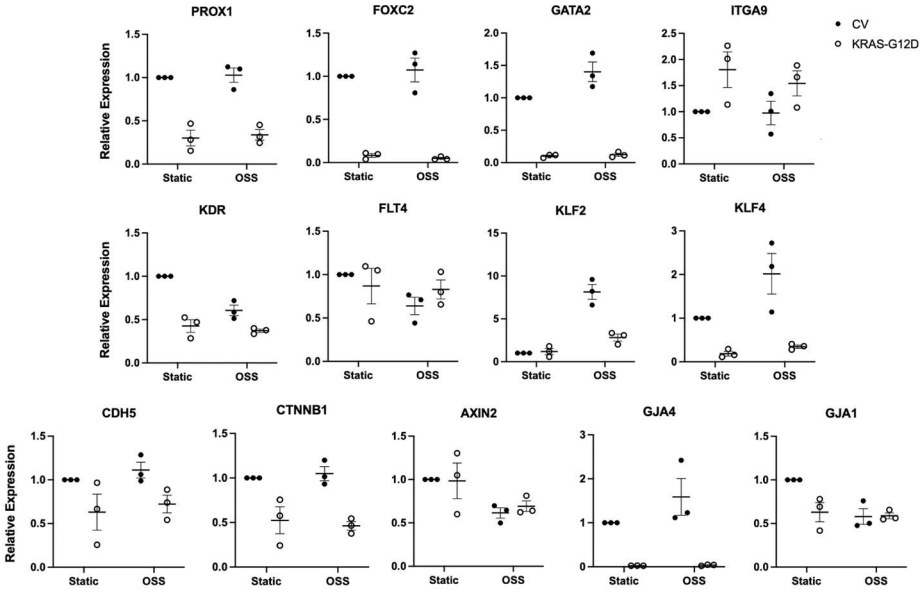

Figure 2. qRT-PCR analysis of cultured LECs infected with a control or KRAS-G12D vector and exposed to static or oscillatory shear stress (OSS) conditions for 48 hours. The expression of many lymphatic valve genes was markedly suppressed when KRAS-G12D was expressed.

Western blot analysis of some of the same genes was performed (**Figure 3**). We show that PROX1, FOXC2, and GATA2 were consistent with the qRT-PCR data and were drastically downregulated. Activated β-catenin, a marker for nuclear β-catenin, revealed that β-catenin signaling was attenuated in the presence of flow. Confirming our preliminary data, VE-cadherin expression was greatly decreased. The only protein that followed a different expression pattern was KLF4, which increased expression in OSS conditions.

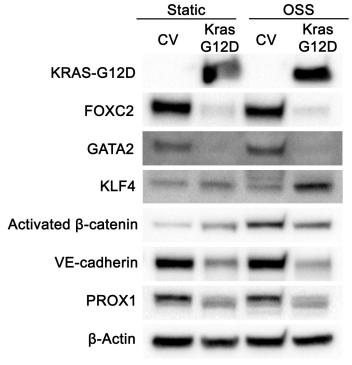

Figure 3. Western blot analysis of some of the genes from Figure 2. Most of the valve proteins were downregulated when KRAS-G12D was expressed.

scRNA-Seq reveals alterations in the cellular landscape of lymphatic vessels in Prox1-CreER^{T2};LSL-Kras^{G12D} mice.

To identify KRAS^{G12D}-induced changes in the cellular and molecular landscape of lymphatic vessels, we analyzed lung LECs from control and *Prox1-CreER^{T2};LSL-Kras^{G12D}* mice by single-cell RNA sequencing (scRNA-Seq). We included the *mT/mG* reporter in our breeding scheme to label LECs with GFP. We fed newborn mice tamoxifen (50 µg; P.O.) from P0 to P2 and digested lungs with a commercially available lung dissociation kit (Miltenyli Biotec; 130-095-927). GFP-positive LECs were sorted by FACS and analyzed by scRNA-Seq using 10x Genomics Chromium 3' chemistry. We then analyzed our data using the Seurat package in RStudio. Our analysis revealed five unique LEC clusters in the lungs of control mice. Based on cluster-specific marker expression the clusters were identified as valve, collecting, mixed, capillary, and Pentraxin 3 (Ptx3) capillary LECs (**Figure 4**). Ptx3 LECs are a recently identified subpopulation of LECs. They are characterized by high expression of immune regulatory molecules and are in the medullary sinuses in lymph nodes and at the blunt ends of lymphatic vessels in the skin. The integrated analysis of control and KRAS^{G12D} LECs revealed that mutant mice have more Ptx3 capillary LECs and fewer collecting and valve LECs than control mice (**Figure 5**). We are continuing to analyze our scRNA-Seq data to understand how hyperactive KRAS signaling impairs the development of lymphatic valves and promotes the expansion of the Ptx3 capillary population of LECs.

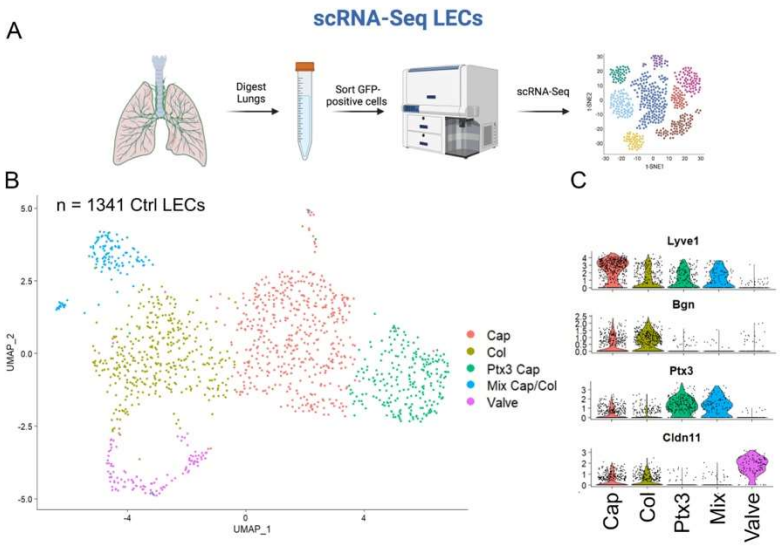

Figure 4. scRNA-Sequencing (Seq) workflow and results for control mice. A. Workflow for scRNA-seq experiment. **B.** UMAP showing the different LEC clusters in control mice. **C.** Violin plots showing the expression of specific marker genes.

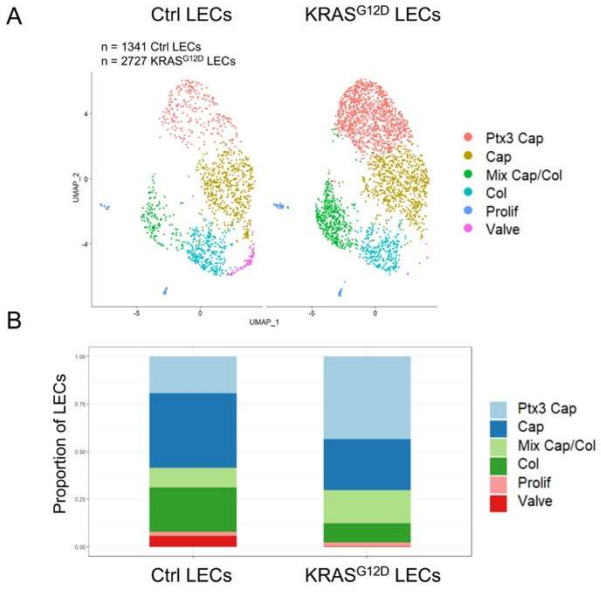

Figure 5. scRNA-Seq results for control and Kras^{G12D} mice. A. UMAP plots for LECs from the lungs of control and Kras^{G12D} mice. **B.** Bar graphs showing the relative proportion of LEC subtypes in the lungs of control and Kras^{G12D} mice.

Aim 2: Test the ability of an FDA-approved inhibitor to prevent and/or reverse phenotypes caused by excessive KRAS signaling in LECs.

Trametinib increases the circularity of KRAS^{G12D}-LECs and suppresses the proliferation and migration of KRAS^{G12D}-LECs.

Trametinib (an FDA-approved MEK1/2 inhibitor) is an emerging treatment for lymphatic anomalies. Trametinib improves patient symptoms (e.g., pulmonary function and pleural effusions/chylothorax) and alters lymph drainage pathways in patients. We previously showed that trametinib attenuates KRAS^{G12D}-induced lymphatic valve loss in neonatal mice. To investigate the effect of trametinib on cellular processes associated with lymphangiogenesis, we treated GFP-LECs and KRAS^{G12D}-LECs with DMSO (vehicle) or trametinib. We found that trametinib-treated KRAS^{G12D}-LECs had more of a standard cobblestone appearance than DMSO-treated KRAS^{G12D}-LECs (**Figure 6**). Circularity index measurements revealed that trametinib-treated KRAS^{G12D}-LECs were significantly more circular than DMSO-treated KRAS^{G12D}-LECs (**Figure 6**). Trametinib also decreased the proliferation and migration of KRAS^{G12D}-LECs (**Figure 6**). These data suggest that MEK1/2 inhibition normalizes aberrant cell behaviors caused by hyperactive KRAS signaling in LECs.

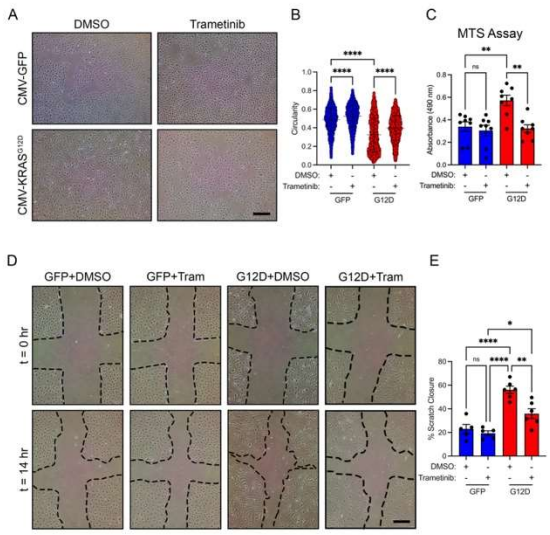

Figure 6. Trametinib decreases KRAS^{G12D}-induced cell shape changes, proliferation, and migration. A. Representative brightfield images of GFP-LECs and KRAS^{G12D}-LECs treated with DMSO or trametinib (10 nM) for 48 hours. **B.** Circularity index measurements for GFP-LECs and KRAS^{G12D}-LECs treated with DMSO or trametinib (10 nM). Trametinib significantly increased the circularity of KRAS^{G12D}-LECs. **C.** MTS viability assay results for GFP-LECs and KRAS^{G12D}-LECs treated with DMSO or trametinib (10 nM) for 72 hours. Trametinib decreased the proliferation of KRAS^{G12D}-LECs. **D.** Representative images of GFP-LECs and KRAS^{G12D}-LECs taken 0 or 14 hours after scratching confluent monolayers of cells. Cells were treated with DMSO or trametinib (10 nM) immediately after scratching. **E.** Graph showing scratch closure area 14 hours after wounding. Trametinib-treated KRAS^{G12D}-LECs closed the scratched area significantly slower than DMSO-treated KRAS^{G12D}-LECs. Data are presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, ns = not significant; ANOVA Tukey's multiple comparisons test. Scale bars = 300 μm.

To characterize the effect of trametinib on cell signaling, we treated GFP-LECs and KRAS^{G12D}-LECs with DMSO or trametinib, and then assessed the phosphorylation of AKT and ERK1/2 by immunoblotting. We found that trametinib significantly decreased ERK1/2 phosphorylation in GFP-LECs and KRAS^{G12D}-LECs (**Figure 7**). Interestingly, trametinib modestly increased AKT phosphorylation in GFP-LECs and significantly increased AKT phosphorylation in KRAS^{G12D}-LECs (**Figure 7**). Trametinib and other MEK1/2 inhibitors have also been shown to induce AKT phosphorylation in a variety of cancer cell lines [34, 35]. Although trametinib affected ERK1/2 and AKT phosphorylation, it did not affect the expression of GFP in GFP-LECs or KRAS^{G12D} in KRAS^{G12D}-LECs (**Figure 7**).

To determine the impact of trametinib on gene expression, we analyzed the transcriptomes of DMSO and trametinib-treated KRAS^{G12D}-LECs by bulk RNA sequencing. We found that 841 genes were upregulated, and 424 genes were downregulated in trametinib-treated KRAS^{G12D}-LECs compared to DMSO-treated KRAS^{G12D}-LECs (**Figure 7**). GO term analysis showed that genes associated with TNF signaling, cell adhesion, integrin binding, and cytokine-cytokine receptor signaling were

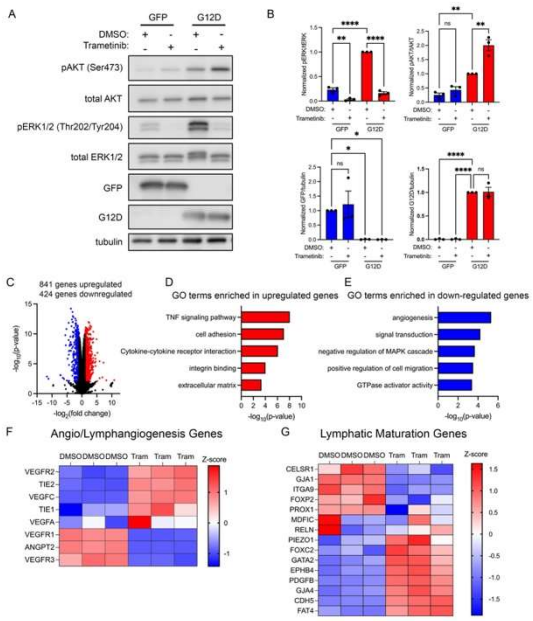

Figure 7. Trametinib decreases MAPK signaling and increases the expression of lymphatic maturation genes. A. Western blot analysis of phospho-AKT, AKT, phospho-ERK1/2, ERK1/2, GFP, $KRAS^{G12D}$ (mutation-specific antibody), and tubulin. Protein lysates were made 16 hours after treating GFP-LECs and $KRAS^{G12D}$-LECs with DMSO or trametinib (10 nM). **B.** Graphs of western blot results. Trametinib increased phospho-AKT levels and decreased phospho-ERK1/2 levels in $KRAS^{G12D}$-LECs. **C.** Volcano plot of RNA-Seq data comparing trametinib-treated $KRAS^{G12D}$-LECs to DMSO-treated $KRAS^{G12D}$-LECs. RNA was isolated 16 hours after treating cells with DMSO or trametinib (10 nM). Eight hundred forty-one genes were upregulated (red dots), and 424 genes were downregulated (blue dots) by trametinib (log_2 fold-change ≥ 1 or ≤ -1; FDR < 0.02). **D,E.** Select GO terms associated with genes upregulated by trametinib (**D**) and genes downregulated by trametinib (**E**). **F,G.** Heatmaps of genes that regulate angio/lymphangiogenesis (**F**) and genes that control lymphatic valve development or lymphatic muscle cell recruitment (**G**). Data are presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, ns = not significant; ANOVA Tukey's multiple comparisons test and Dunnett's multiple comparisons test.

upregulated by trametinib (**Figure 7**). Additional GO term analysis demonstrated that genes associated with angiogenesis, GTPase activity, and cell migration were downregulated by trametinib (**Figure 7**). Trametinib also decreased the expression of negative regulators of the MAPK pathway (**Figure 7**). We then examined the expression of genes that promote angio/lymphangiogenesis and genes that regulate the maturation of lymphatic vessels. We found that trametinib decreased the expression of several angio/lymphangiogenic genes, and increased the expression of several genes that promote the maturation of lymphatic vessels (**Figure 7**).

Trametinib suppresses $KRAS^{G12D}$-induced lymphatic vessel hyperplasia.

We previously showed that trametinib suppresses the loss of lymphatic valves in newborn mice that express $Kras^{G12D}$ in their LECs. We did not assess the effect of trametinib on the diameter of lymphatic vessels. Evaluating the impact of trametinib on the size of lymphatic vessels is clinically relevant because patients with RAS pathway mutations have dilated lymphatic channels. To assess the effect of trametinib on lymphatic hyperplasia in $LEC^{KrasG12D}$ embryos, we treated embryos with vehicle or trametinib from E11.5 to E13.5 and stained back skin from E14.5 embryos with an antibody did not significantly affect the number of lymphatic branch points in $LEC^{KrasG12D}$ embryos (**Figure 8**). However, trametinib significantly decreased the diameter of lymphatic vessels in $LEC^{KrasG12D}$ embryos (**Figure 8**). These data suggest that pharmacologic inhibition of MAPK signaling can partially suppress $Kras^{G12D}$-induced lymphatic hyperplasia *in vivo*.

Genetic inhibition of MAPK signaling in LECs partially suppresses $KRAS^{G12D}$-induced lymphatic vessel hyperplasia.

Trametinib blocks MEK1/2 activity throughout the body. To determine whether specifically blocking MAPK activation in LECs could suppress $KRAS^{G12D}$-induced lymphatic vessel hyperplasia, we obtained $LSL\text{-}Map2k1^{K97M}$ transgenic mice. $LSL\text{-}Map2k1^{K97M}$ transgenic mice express a dominant-negative form of human MEK1 following Cre-mediated removal of an upstream transcriptional stop sequence (**Figure 9**). The K97M mutation abolishes MEK1's kinase activity but does not affect its

ability to interact with ERK1 and ERK2. To assess the effect of the Map2k1^{K97M} mutation on lymphatic vessel development, we collected back skin from E15.5 embryos and stained it for neuropilin-2 (**Figure 9**). We found that the number of lymphatic vessel branch points was not significantly different between *LEC^{Ctrl}* and *LEC^{Map2k1K97M}* embryos. The number of branch points was also not significantly different between *LEC^{KrasG12D}* and *LEC$^{KrasG12D;Map2k1K97M}$* embryos (**Figure 9**). The diameter of lymphatic vessels was slightly lower in *LEC^{Map2k1K97M}* embryos compared to *LEC^{Ctrl}* embryos. However, this did not reach statistical significance. In contrast, lymphatic vessel diameter was significantly smaller in *LEC$^{KrasG12D;Map2k1K97M}$* embryos compared to *LEC^{KrasG12D}* embryos (**Figure 9**). These results suggest that blocking MAPK activation in LECs partially suppresses KRAS^{G12D}-induced lymphatic vessel hyperplasia.

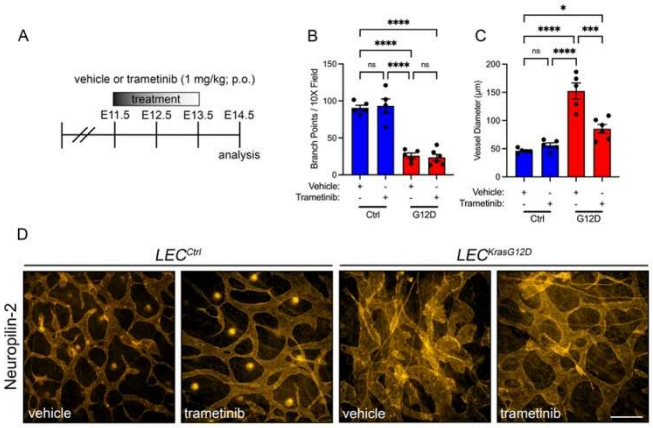

Figure 8. Trametinib partially suppresses Kras^{G12D}-induced enlargement of lymphatic vessels. A. Schematic showing when mice received vehicle or trametinib (1 mg/ml; p.o.; q.d.). **B.** Lymphatic vessel branch points. **C.** Lymphatic vessel diameter measurements. **D.** Back skin whole-mounts from E14.5 embryos stained for neuropilin-2. Data are presented as mean ± SEM. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$, ns = not significant; ANOVA Tukey's multiple comparisons test. Scale bar = 250 µm.

What opportunities for training and professional development has the project provided?

The project has provided numerous opportunities for Dr. Fernandes, Mr. Tresemer, Ms. Mastrogiacomo, and Dr. Jannaway to learn tissue culture, molecular biology, and histology/imaging techniques. Dr. Jannaway completed her postdoctoral fellowship in Dr. Scallan's lab and is now a Senior Scientist at AstraZeneca in Cambridge, England. The Dellinger and Scallan laboratories have a joint lab meeting 1-2 times a month. This meeting allows trainees and staff to present their research, discuss papers, and gain in-depth knowledge about LMs. It also enables trainees and staff to receive mentorship from both Drs. Dellinger and Scallan. Members of the Dellinger and Scallan laboratories regularly attend an international seminar series on lymphatic/vascular malformations led by Dr. Dellinger. This seminar series also allows trainees and staff to learn more about the treatment and diagnosis of LMs and the latest advances in clinical and basic science research on LMs.

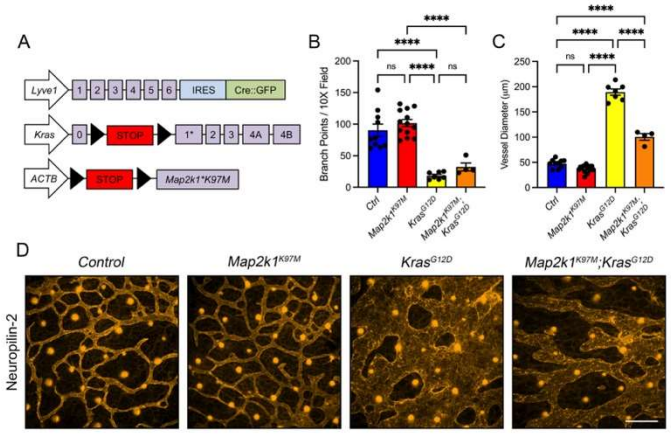

Figure 9. A dominant-negative form of MEK1 (Map2k1^{K97M}) partially suppresses Kras^{G12D}-induced enlargement of lymphatic vessels. A. Schematics of the *Lyve1-Cre*, *Kras$^{LSL-G12D}$*, and *Tg$^{LSL-Map2k1K97M}$* alleles. **B.** Lymphatic vessel branch points. **C.** Lymphatic vessel diameter measurements. **D.** Back skin whole-mounts from E15.5 embryos stained for neuropilin-2. Data are presented as mean ± SEM. **** $P < 0.0001$, ns = not significant; ANOVA Tukey's multiple comparisons test. Scale bar = 250 µm.

How were the results disseminated to communities of interest?

Drs. Dellinger and Scallan have presented results from this project at several scientific meetings (see **Section 6: Products**).

What do you plan to do during the next reporting period to accomplish the goals?

During the next year, we will finish analyzing and validating our scRNA-Seq data. We will also assess the effect of trametinib on *iLEC*^{KrasG12D} mice with established disease and the durability of responses to trametinib. We will also assess how KRAS^{G12D} expression affects the contractile activity of collecting lymphatic vessels. The expression patterns of valve genes will be assessed *in vivo* by whole mount immunofluorescence.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We have found that the FDA-approved MEK1/2 inhibitor, trametinib, blocks MAPK signaling and the proliferation and migration of KRAS^{G12D}-LECs. It also increases the expression of several genes that regulate lymphatic valve development. We also show that trametinib and Cre-mediated expression of a dominant-negative form of MEK1 (*Map2k1*^{K97M}) suppresses KRAS^{G12D}-induced lymphatic vessel hyperplasia in embryos. Together, our data indicate that KRAS/MAPK signaling must be tightly regulated during embryonic development for the proper development of lymphatic vessels and further support the testing of MEK1/2 inhibitors for treating lymphatic malformations.

What was the impact on other disciplines?

KRAS is an oncogene frequently mutated in cancer and arteriovenous malformations (AVMs). Our large transcriptomic data sets for KRAS^{G12D}-LECs could help shed light on the molecular mechanisms by which hyperactive KRAS signaling promotes cancer and AVMs.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. The following has been accepted for publication and is in press.

  Fernandes LM, Tresemer J, Zhang J, Rios JJ, Scallan JP, Dellinger MT. Hyperactive KRAS/MAPK signaling disrupts normal lymphatic vessel architecture and function. *Frontiers in Cell and Developmental Biology*. 2023.

  Books or other non-periodical, one-time publications.

  Nothing to Report.

Other publications, conference papers and presentations.

Michael Dellinger, Ph.D.
American Society of Human Genetics
October 25th – October 29th, 2022
Los Angeles, CA

Michael Dellinger, Ph.D.
Gordon Research Conference: Lymphatic Vessels as Multifaceted Regulators of Health and Disease

October 30th – November 4th, 2022
Tuscany, Italy

Michael Dellinger, Ph.D.
Vascular Anomalies Cure Network International Conference
January 31st – February 3rd, 2023
Brussels, Belgium

Michael Dellinger, Ph.D.
5th Annual Gulf Coast Vascular Biology Research Consortium
August 4th – 5th, 2023
College Station, TX

Michael Dellinger, Ph.D.
International Congress of Lymphology
September 11th – 15th, 2023
Genoa, Italy

Michael Dellinger, Ph.D.
International Scientific Conference on Complex Lymphatic Anomalies
September 29th – 30th, 2023
Dallas, TX

Joshua Scallan, Ph.D.
International Scientific Conference on Complex Lymphatic Anomalies
September 29th – 30th, 2023
Dallas, TX

- **Website(s) or other Internet site(s)**

  Nothing to Report.

- **Technologies or techniques**

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**

  Nothing to Report.

- **Other Products**

  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Michael Dellinger
Project Role:	PI
Researcher Identifier:	ORCID ID: 0000-0002-3315-4239
Nearest person month worked:	2.4
Contribution to Project:	Dr. Dellinger is responsible for overseeing the planning, performance, and interpretation of experiments. He is also responsible for preparing manuscripts, reports, and ensuring all aspects of the project are compliant with UTSW and DOD policies.
Name:	Lorenzo Fernandes
Project Role:	Research Scientist (Dellinger Lab)
Researcher Identifier:	ORCID ID: 0000-0001-9727-7847
Nearest person month worked:	6
Contribution to Project:	Dr. Fernandes is responsible for data acquisition and analysis.
Name:	Jeffrey Tresemer
Project Role:	Research Assistant I (Dellinger Lab)
Researcher Identifier:	ORCID ID: 0000-0001-9727-7847
Nearest person month worked:	12
Contribution to Project:	Mr. Tresemer is responsible for managing the animal colony, genotyping mice, and administering drugs to mice.
Name:	Joshua Scallan
Project Role:	PI
Researcher Identifier:	orcid.org/0000-0002-1190-7308
Nearest person month worked:	3.6
Contribution to Project:	Dr. Scallan is responsible for planning and interpreting experiments, managing breeding of mice, and training personnel on this project. He also prepares manuscripts, progress reports, and ensures that all aspects of our research are compliant with the USF and DOD policies.
Name:	Melanie Jannaway
Project Role:	Research Associate
Researcher Identifier:	orcid.org/0000-0002-4390-9623
Nearest person month worked:	3

Contribution to Project:	Dr. Jannaway is responsible for breeding mice, collecting tissues, immunostaining, cell culture, data analysis, performing *ex vivo* lymphatic permeability and contractile function analyses, and making figures for publication.
Name:	Diandra Mastrogiacomo
Project Role:	Graduate Student
Researcher Identifier:	orcid.org/0000-0002-1148-5926
Nearest person month worked:	12
Contribution to Project:	Ms. Mastrogiacomo is a new graduate student that joined Dr. Scallan's lab in August 2022 and will be responsible for breeding mice, injecting tamoxifen, collecting tissues, immunostaining, cell culture, data analysis, and making figures for publication. She will also prepare any manuscripts arising from the project.
Name:	Kunyu Li
Project Role:	Research Technician
Researcher Identifier:	https://orcid.org/0000-0003-0082-2883
Nearest person month worked:	12
Contribution to Project:	Ms. Li is responsible for maintaining the mouse colony by tattooing mice, collecting tails snips for DNA extraction, performing genotyping PCRs, running DNA electrophoresis gels, and weaning mice.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Source: NIH/NHLBI 1R01HL164825-01 (PI: Scallan, Joshua)
Project title: VEGFR Signaling Controls Lymphatic Junctions
Project period: 7/1/2022 - 6/30/20226
Goals/specific aims: The goal of this grant is to investigate the role of VEGFR3 in the lymphatic vasculature. In Aim 1 we will assess the ability of lymphatic capillaries to remodel their junctions in the absence of VEGFR3 at various timepoints after birth. We will also investigate whether VEGFR3 is required not only for button junction formation, but also for the lifelong maintenance of these special junctions. Lymph flow will be assessed in vivo to determine how the loss of button junctions affects physiological interstitial fluid absorption. In Aim 2, we will investigate the downstream cell signals that regulate button junction formation and identify the signaling pathways involved using a variety of approaches.
Potential Overlap: None

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.