The EHE Foundation (USA)
The EHE Rare Cancer Charity (UK)
The EHE Rare Cancer Foundation (Australia)
EHE Italia - Non solo Laura
EHE Canada

Quarterly Newsletter for the EHE Group
January - March 2022

the pledge

Edition 28

Contents

Welcome

Welcome to our 28th edition of **The Pledge,** the quarterly newsletter of the EHE Group, for the first quarter of 2022. As always, this newsletter includes updates on all our main activities and areas of focus, and in particular patient support and advocacy activities, fundraising, and research.

We hope you will enjoy reading about what our global community are doing. As always, we also want to say a huge thank you to all our supporters for their contributions. ***"Just Live".***

Highlights

The 2022 EHE 360 Conference is a huge successs

The 2022 EHE 360 International Conference, organised by The EHE Foundation, was another huge success. Read about the conference and see important links in the dedicated EHE 360 section of this edition of The Pledge.

The EHE Foundation in the US celebrates its 7th birthday

The EHE Foundation, first of the EHE foundations established in 2015, had its seventh birthday this quarter. What a seven years it has been, with so much achieved.

Research accelerates in the search for TEAD inhibitors

Excitement has been rising in the EHE community because of the growing focus on TEAD inhibitors which have the potential to be game changing in the treatment of EHE. Several new drugs are under development, and some clinical trials are underway.

Positive research results in two UK research projects

Positive progess has been reported from the development of a zebrafish EHE model at the Bateson Centre at the University of Sheffield and from the PhD at the University of Manchester which is evaluating how the fusion protein impacts our cells.

Grassroots fundraising continues to expand post COVID

Once again we have seen fantastic support from our grassroots supporters, driving fundraising for EHE research which is so important, particularly following the last two years of COVID pandemic.

Further details on these stories, and much more, can be found in this edition

the pledge

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Just Like

2022 EHE 360 International Conference

An amazing coming-together of EHE researchers, clinicians, patients and advocates from around the world!.

The 2022 EHE 360 International Conference was a great success. Held virtually over two days in January, the event brought together the global EHE community. It was so incredible to see 367 participants from around the world registered!

The EHE 360 Conference is organized by The EHE Foundation (US) working with global partners, including The EHE Rare Cancer Charity (UK) and The EHE Rare Cancer Foundation (Australia) bringing together researchers, clinicians, patients, and advocates from around the world to advance the fight for treatments and a cure for EHE. The EHE Foundation Executive Director, Medha Deoras-Sutliff said:

> "We are incredibly grateful to everyone who contributed to making this event such a success! It was an absolute global team effort and the actions and collaborations resulting from the EHE 360 initiative are proving to be game-changing in pushing forward research efforts to effective treatments for EHE".

The conference objectives:

This year's conference had three main goals:

- **EDUCATION:** Increasing knowledge and understanding of key issues central to the diagnosis, treatment, and cure for EHE
- **INNOVATION:** Providing a novel, catalytic framework to identify gaps and foster dialogue in basic, translational, and clinical research of EHE
- **COLLABORATION:** Stimulating an interdisciplinary sharing of clinical best practices by strengthening and supporting global collaboration world-wide

EHE 360 Conference Highlights

The EHE Foundation Board President, Jenni Kovach, welcomed attendees from around the world. Back by popular demand, EHE Foundation Board member, Dr. David Casimir, reprised his role as the conference moderator.

Day one featured the Scientific Symposium with seven science sessions, including 24 presenters from around the world. Hugh Leonard commented to researchers during the final session of the day:

> "I find it absolutely staggering to think where we were in 2015-16 where if we'd had this conference, we probably would have had 1 or 2 people presenting, and today it is really tremendous what you (researchers) are achieving. Thank you very much!".

Over 150 clinicians and researchers from around the world registered to attend!

Scientific Symposium Sessions included:

- EHE Biology & What We Know
- EHE Models
- Targeting TAZ-CAMTA1 in EHE
- Gene Fusions in EHE
- Modulation of YAP/ TAZ in EHE
- Phase I Clinical Trial Spotlight – Ikena Oncology
- Roundtable - Advancing Translational & Transformational Research in EHE

2022 EHE 360 International Conference

Day two featured the **EHE Global Patient Conference** designed specifically for patients and their families to bring patients, clinicians, advocates and researchers together. Presenters explored diverse themes, EHE challenges and identified needs critical to EHE patients and caregivers. Denise Robinson, Director of Research and part of the Conference planning team commented:

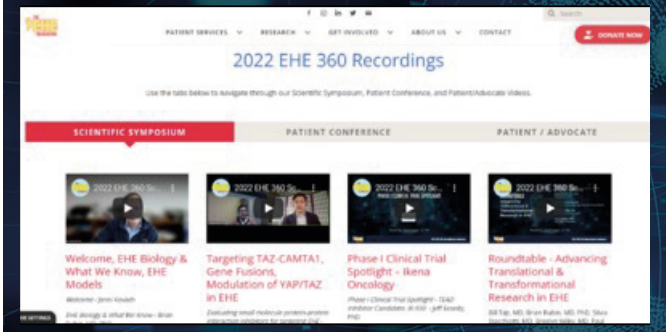
> "hope was the greatest take-away from this year's conference – it is the fuel we all need, and the presenters have so generously given. We see tangible progress from world-class and emerging researchers, clinical trials are coming forward, and we can feel the energy and passion coming from the clinical community to find answers, and help patients fight this disease."

The Patient Conference included:

- Introduction to ESMO Consensus Paper
- Spotlight on EHE Researchers
- Biobanking – Patient Participation Advancing EHE Research
- Accessing and Understanding Specialty & Multi-Disciplinary Care
- Ask The Experts

View conference sessions:

All conference sessions, comprising 9 hours of excellent content, can be viewed on The EHE Foundation website at **https://fightehe.org/2022-ehe-360/**, including excellent patient advocate messages from members of our EHE community.



Many thanks to the Co-Chairs and Conference Leaders

Planning for the 2022 EHE 360 Conference began almost immediately after the 2021 EHE 360 Conference ended a year ago. Drs. Bill Tap, Brian Rubin, and Silvia Stacchiotti each graciously accepted the role of 2022 Conference Co-Chairs. Their guidance, with the efforts of a conference Steering Committee, helped The EHE Foundation successfully execute this year's impactful event.

Huge thanks also to our financial supporters

The Foundation was delighted to secure Conference sponsors who generously provided funds that offset event planning expenses.

The Foundation is extremely grateful for this wonderful support:

Platinum Sponsor:
Ikena Oncology

Silver Sponsor:
EveryLife Foundation

Bronze Sponsor:
Cleveland Clinic Sarcoma Program

The EHE Foundation is also deeply grateful to the Margie and Robert E. Petersen Foundation and the Chan Zuckerberg Initiative's Rare As One Project for their continued funding and support.

EHE 360 by the Numbers

- 367 people registered to attend
- 30 countries were represented by attendees
- 216 unique attendees participated over the 2-day event
- 39 presenters gave their time to present or participate in discussions

01 Patient Support and Advocacy

The EHE Group's patient support is almost entirely driven by the boundless energy and contribution of so many of the members of the global EHE community.

These are the people that continue to warmly and compassionately welcome and support anyone diagnosed with EHE, wherever they may live. It is for the EHE community that our EHE Group works so hard to deliver increased awareness of EHE, while striving to create ever-greater support and connectivity for our members. We thank all our supporters who have contributed to this critical part of our activity, examples of which are provided in this section.

Scanxiety

This past quarter, The EHE Foundation spent some time talking about scanxiety on social media. It's a common topic for patients and caregivers and is especially true due to the unpredictable nature of EHE..

What is scanxiety?

Scanxiety describes the apprehension felt by people with cancer as they wait for their next scan.
It's a form of anticipatory anxiety. Scans are like emotional roulette. You're trying really hard not to worry about the unknown, but your body has other plans.

Recognizing the Signs of Scanxiety

Symptoms of scanxiety vary from person to person. Here are a few things you might be experiencing.

- increased heart rate
- trouble sleeping
- tension and irritability
- stomach pain/nausea/appetite loss
- sweating
- difficulty concentrating
- trouble regulating emotions and/or mood
- emotional numbness
- umpiness or restlessness
- racing thoughts and panic

Symptoms range from mild to intense and can be invasive to your daily life. You just don't feel like yourself and normal activities become cumbersome and trying. It becomes hard to make decisions until after your scans have passed.

What can you do to manage scanxiety?

Most importantly, know that your feelings are valid, and you are not alone. These feelings are very typical for people dealing with a difficult diagnosis. To cope with your scanxiety, you'll need to recognize your symptoms, and make a plan.

Make note of the symptoms that you typically feel and the go-to coping strategies that work for you. Keep your plan handy for when scanxiety creeps in.

Three Strategies for Coping with Scanxiety

1. Mindfulness

Mindfulness is about accepting what is, accepting that unpleasant feelings are happening, and riding it out with as little judgment of whether those feelings are right or wrong, or whether or not you should be feeling them. They are happening. Period.

2. Contact Your Support Structure

Reach out to family and friends that ground you and provide steady, calm advice in times of worry. Some patients may prefer support groups or professional counsellors.

3. Relax with Intention

Breathwork, meditation, massage, yoga or light exercise, journaling, reading, listening to a book, or focusing on a hobby are all ways to use relaxation to cope with scanxiety.

While the symptoms and coping techniques vary from person to person, patients with a serious diagnosis almost inevitably feel anxiety surrounding their condition. It's important to plan for when this happens. We can't prevent all scanxiety, but we can mindfully confront it. A little plan can help in a big way!

Happy Birthday to the EHE Foundation

In 2015, The EHE Foundation officially became a non-profit organization. Julie Wahl was excited to share her thoughts as the foundation turned 7 years old:

> "Happy Birthday to The EHE Foundation! I can't believe it has been 7 years! So many people have contributed to the extraordinary progress we've made over the last 7 years. Our volunteers and Board Members have given thousands of hours of their valuable time. Patients have contributed to research, awareness, and the support of each other. Doctors, researchers, and our very own Advisory Board are collaborating more than ever. Donors have repeatedly supported our efforts to fund EHE research. Together, we can be proud of what we've accomplished."

Here are some highlights of the accomplishments of the last 7 years, including the research that the EHE Foundation and its sister groups have supported:

- Over $7m raised
- Research being funded in multiple institutions
- A greater understating of EHE biology being developed
- Fusion protein identified
- Genetically-engineered mouse delivered
- New therapeutic targets being researched
- TEAD inhibitors under development
- PDX mice established
- Rare as One membership secured
- Foundation infrastructure being funded
- Two EHE international conferences held
- Sister foundations set up in the UK, Australia, Italy and Canada
- Zebrafish model progressing

The Board and Management Team of the EHE Foundation want to thank all those who have contributed so much:

01 Patient Support and Advocacy

> “Thanks to the amazing effort of everybody who works so tirelessly in the EHE Group, in support of our global EHE patient community. These are the people who face and deal with their own EHE journeys, and who inspire us and motivate us everyday with their courage and positivity. We also want to say thank you to our brilliant grassroots supporters who have raised so much money. We also need to recognize the immense contributions received from the Margie and Robert E. Petersen Foundation and the Chan Zuckerberg Initiative, both of which have been transformational for our EHE program. Finally, a huge shout-out for all the clinical specialists who care for our patient members, and the researchers who are working so hard to find a cure for EHE. Thank you everybody for making the last 7 years so successful. We know there is a lot more to do!”

The EHE Facebook community continues to grow

As we said good bye to 2021, Lisa Hartle De Young shared information about the growing size of the group. With 2188 members representing 77 countries across the globe, the EHE Facebook page is a unique resource for such a rare cancer, allowing us to access key information and data to help understand the disease.

Of course the EHE Facebook page is a closed site, due to the confidential nature of the content, and so all new applicants pass through a screening process, to understand what their connection to EHE is. This is a time consuming but important process, with Lisa Hartle De Young and Julie Rivers Wahl administering the process. In 2021 Lisa processed nearly 500 membership requests in the year, adding 233 new members, as well as EHE patients who are not on social media.

We want to thank Lisa and Julie for their fantastic work in administering and helping new patients join the group. You guys are true stars.

A change in perspective after a cancer diagnosis

Receiving an EHE diagnosis is shattering. The first few months for many a new patient are a rollercoaster of scans, appointments, worries and anxiety. But many of our members also find that their perspectives on life change as they begin to come to terms with their situation. Tonja Mobley Pektas posted just such a message.

> “Today is my first day in my fifties! I used to think that getting older was depressing...the fine lines, extra pounds...you know. But approaching a year after my diagnosis and I now see birthdays as a celebration! Bring on the wrinkles because that means I’m getting older and every day is a gift! I now see growing older as a huge blessing! Crazy how this illness can change your perspective and make you appreciate each day! ***Just live,*** my friends!”

Thank you for sharing that message Tonja. We hope you had a great birthday and may you enjoy many more, even if they do come with a few more pounds and a few more wrinkles!

Calling all European EHE patients

Mariana Coutinho started 2022 by reaching out to update our European EHE patients with the news of the European EHE awareness campaign.

> "Hi everyone! Some of you may remember a post that I made last year, asking if there was anyone from Europe interested to help with an awareness campaign about EHE. Well, I am glad to share that, so far, 32 of you (from 15 different European countries!) have come forward and expressed that you are ready to help, and for that we are extremely grateful. Therefore, I am once again writing this post in case any of you missed the last post and would be interested to join us."

The objective is to develop a pan-European EHE-focused patient federation. The EHE Rare Cancer Charity in UK (EHERCC) wants not only to connect with all current EHE patients from Europe in our EHE family but also wants to reach out to even more EHE patients in each country. Mariana continued:

> "This connectivity in such a rare disease is important as it will hopefully make it easier to improve research and the care we receive while living in Europe, and will help us ensure that EHE patients get the maximum exposure to any future study or research or clinical trial opportunities. We hope that it will also expand the support network between patients across Europe through social media. This may not be for everybody, but we see many patients who find this form of connectivity very helpful."

Additionally, EHERCC will be reaching out to different cancer centres/hospitals across Europe to let healthcare professionals know about EHE and how to effectively support EHE patients. They hope that they can do this effectively following the ESMO EHE Consensus Paper that was published in 2021 and involved clinical specialists of many different disciplines from many different countries across Europe.

So having identified many European patients who are keen to be involved, the EHERCC will soon be making contact with updates on their growing European position and organisation. They are also hoping to organise regular videoconference calls amongst the group.

If you are interested in participating in these growing European initiatives, please e-mail your details to:

1. Mariana Coutinho at:
   mariana_coutinho@live.com.pt and/or
2. Sally Baker at:
   sbaker1339@gmail.com and/or
3. Hugh Leonard at:
   hleonard@ehercc.co.uk

01 Patient Support and Advocacy

Sleep and Cancer

> "I know many EHE patients are interested in healthy approaches. We don't talk about sleep enough, and it's so important. Sleep and cancer are discussed around 34 minutes in to this podcast. Sleep is vital for your immune system to work properly,"

said Lael Bellamy.

It certainly is a fascinating listen, and the discussion is kept relatively simple.

Thank you, Lael, for sharing this important information. For those interested you can find this podcast at: **https://www.youtube.com/watch?v=pv7i22DHiVE**

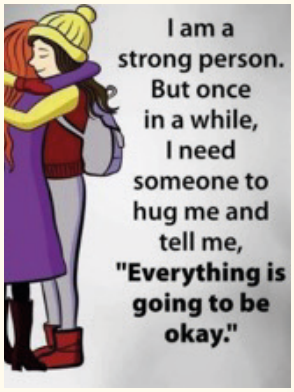
Hugging is to be encouraged

One of the things that there is a lot of in our Facebook pages, and amongst the EHE Foundation participants, is hugging. Hugh Leonard is a strong advocate of hugging and explains why:

> "There is something very special about hugging. Hugs are given and received in love and friendship. The act of hugging actually places you in a vulnerable position, with your arms thrown out wide, so it is also a sign of real trust in the target of your hug. And the actual sensation of being hugged is so special. It is common to almost every society, and is used at moments of spontaneity and extreme emotion, be that love, fear, or sadness. When you see two people hugging, you know immediately something special is happening. So I would encourage everybody to hug lots, hug long and hug hard. Wrap your arms around the people you love, who mean a lot to you or have done something really special, and hold on"

Carl Dixon was also championing hugging:

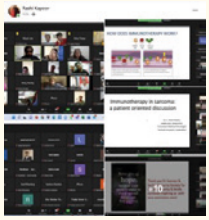
> "This is not only for those of us with EHE but for all the caregivers and family members, as we all need a hug now and then. We can see what the person with EHE is going though and how they suffer but far too often we do not see what the caregiver is going through, their pain is also real. It's not always a physical hug but words can also provide that much needed comfort."



Sachin Sarcoma Society holds patient day

The EHE Group engages with several other sarcoma patient support groups around the globe. One of these is the Sachin Sarcoma Society in India. This group, supporting sarcoma patients, including EHE, in India, held its 98th virtual patient support group meeting for sarcoma patients and caregivers in the first quarter..

> "The meeting was organised for sarcoma patients and joined by 53 patients from all parts of India. An enriching and educational presentation on Role of immunotherapy in sarcomas was given by Dr Rohit Reddy , Medical Oncologist, Yashoda hospital Hyderabad and was followed by an interactive question and answer session."



Dr Reddy addressed a number of issues raised by the patients attending the meeting. These included what immunotherapy is, how it works, what drugs are used, the side-effects of the drugs etc. Everybody agreed that this was a very useful meeting and answered many questions.

The second part of the meeting involved a number of different participants addressing a range of diverse issues, including the side effects of chemotherapy, yoga for deep breathing to assist with stress and anxiety, strategies for caregivers to assist in managing their roles, patient journeys and the difference between targeted and chemotherapy.

We congratulate all those who took part for their time and diligence in holding such a successful day for patients and caregivers, and look forward to working closer with them in the future.

Thanks to a wonderful woman

Our regular readers and those on the EHE Facebook page will know that Carl Dixon is a frequent contributor to the group. This quarter was no different, with some of Carl's posts focusing on the carers of those with EHE, and in particular for Carl, his wonderful wife Elizabeth.

> "This is for my wife and for all the Caregivers out there. We with EHE do not fight this fight alone, thank you"

Carl wanted to share a special song "My Woman, My Woman, My Wife" which he felt said a lot, especially for Carl himself. Carl explained:

> "My wife is the rock, the foundation of our family. Just three years after we were married our life took a path so different to anything we could have imagined. I may have been the one with cancer but this battle was and is a fight both of us were propelled into. Post diagnosis my young wife had to take control of running nearly every daily aspect of our lives all while becoming a caregiver which many times is a 24/7 job. For these past 22 years she has been the concrete that has held this family together, kept me alive by being my medical advocate; my trainer; my researcher; my kick-in-the-ass on down days; mother to our children; teacher to our children; family organizer; driver to our kids as my blindness does not allow me to drive; she is a hockey team manager in support of our children; she holds down a full time job; and this list could go on & on with all the selfless things she does for me, our children and others. If a Medal of Honor could be given to a wife and a mother for stepping up and sacrificing all her dreams in life to selflessly care for her terminally ill husband, my wife has earned one for sure. I could never thank her enough, I could never give her the reward she has earned. She has sacrificed so much here on earth and like a line in this song says if I have earned a slot in heaven I would give it to her. ***Just Live*** my Friends."

We want to thank Carl for once again shining a light on all our caregivers, wherever they are in the world, who so selflessly support loved ones dealing with an EHE diagnosis. We, too, want to say a special thank you to Elizabeth for all she does and for all she contributes to our group.

02 EHE Research

Our pipeline of new EHE proposals seeking funding and support continues to provide exciting opportunities for new projects to add to our existing EHE research. In this edition of The Pledge we are delighted to be able to report on both ongoing and new research funded by the EHE Group. This leaves us excited by the potential to deliver improvements in the care and treatment of EHE patients everywhere.

A New Phase of EHE Research

January brought lots of new excitement to the EHE community as we officially learned about not just one, but two new Phase 1 clinical trials specifically including EHE patients. Phase I studies are the first step in testing new drug candidates in humans. These studies are done primarily to test the safety, side effects, and the best dose of a potential new treatment. In Phase I studies some study participants may benefit from the drug candidate being tested; however, disease response is not the primary purpose of Phase 1 studies. Denise Robinson, Director of Research for The EHE Foundation (US) commented:

> **"We are delighted that the pharmaceutical industry is investigating these new therapeutic candidates. These studies represent meaningful steps toward our mission to find effective treatments and a cure for EHE."**

Ikena Oncology Phase I Study of TEAD Inhibitor Candidate, IK-930

The EHE Foundation were delighted to welcome Ikena Oncology to the EHE360 international EHE conference in January, where they presented their Phase I (first-in-human) clinical trial of their TEAD inhibitor candidate, IK-930, for the treatment of cancers harbouring genetic mutations in the Hippo pathway.

IK-930 is designed to selectively bind TEAD and to disrupt TEAD-dependent transcription of key genes involved in cancer progression, metastases, and therapeutic resistance. Preclinical research, including data presented by Ikena at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics in October 2021, suggests that IK-930 is a potent and selective TEAD inhibitor that could prove effective both as a monotherapy and in combination with multiple targeted agents, such as EGFR and MEK inhibitors, in multiple hard-to-treat cancers.

This study is taking place in the United States. A detailed overview of the study centers conducting this trial, contact information, and inclusion criteria for participants can be found online at **https://clinicaltrials.gov/ct2/show/NCT05228015.**

Novartis Pharmaceuticals Phase I Study of IAG933

Novartis has initiated a global Phase I (first-in-human) clinical trial of IAG933, a YAP/TEAD inhibitor, in patients with mesothelioma, NF2/LATS1/LATS2

ClinicalTrials.gov Identifier	Study Title / Phase	Location(s)	Principal Investigator
NCT04028063	Doxorubicin Plus Dual Checkpoint Blockade for Soft Tissue Sarcomas - Phase 2	Aurora, Colorado	Breelyn Wilky, MD
NCT04784247	Lenvatinib and Pembrolizumab in People with Advanced Soft Tissue Sarcoma - Phase 2	NY & NJ Locations	Sandra D'Angelo, MD
NCT03331250	Eribulin in Angiosarcoma and Epithelioid Hemangioendothelioma (EHE) - Phase 2	Boston, MA	Gregory Cote, MD, PhD
NCT04577014	INCMGA00012 (Anti-PD-1 Antibody) With Gemcitabine and Docetaxel in Patients with Advanced Soft Tissue Sarcoma - Phase 1/2	New York, NY	Sandra D'Angelo, MD

mutated tumors and tumors with functional YAP/TAZ fusions. Patients with malignant EHE can be enrolled with only histological confirmation of the disease.

This study is taking place in the US, Australia, Germany, Italy, Japan and Spain. A detailed overview of the study centers conducting this trial, contact information, and inclusion criteria for participants can be found online at **https://clinicaltrials.gov/ct2/show/NCT04857372. org/ehe-clinical-trials/**. In addition, there are many other studies investigating treatment for sarcomas listed on **www.clinicaltrials.gov.**

Zebrafish progress is exciting

Four years ago, the EHE Rare Cancer Charity in the UK contracted with The Bateson Centre at the University of Sheffield to initiate research into the development of a zebrafish model of EHE. The Bateson Centre is the oldest and largest zebrafish facility in Europe, with substantial experience in the development of zebrafish models of human diseases.

The project started in 2018 and although initially good progress was made, a substantial problem arose, with the research team unable to get the EHE translocation construct provided by Dr Brian Rubin to appear in endothelial cells. Despite attempting several different methods, getting the construct into the endothelial cells remained frustratingly elusive. As a result, at the start of 2020, the research team, together with EHERCC personnel, undertook a detailed project review, including Dr Fredericus van Eeden reaching out to different specialists and researchers to discuss the situation.

This review was protracted by the peak of the COVID pandemic through the year, but eventually identified some very interesting proposals to address the situation.

In mid-2021, the EHERCC contracted to fund a further year of research to provide the research team with the time needed to test the new ideas. This work has been time consuming as the researcher has attempted a number of new ideas, including the painstaking breakdown of the construct into its constituent parts in order to try and identify what is blocking the construct from appearing in endothelial cells.

So the EHERCC team were excited when Dr Van Eeden emailed some good news in February:

02 EHE Research

> "I am very excited because we now have identified 2 independent transmitters of our "switchable" transgenic where TAZ CAMTA can be driven by a new promoter ubi. Eleanor just sent me this photo 10 mins ago where you can see the "detection marker" in red in the picture below."

This marker is attached to the construct and so everywhere you see red, the construct is present. It appears everywhere in the fish, including the endothelial cells. Dr Van Eeden went on:

> "This means that if all is well, we are just a few steps away from expressing TAZ-CAMTA1 in zebrafish! We are now first securing the line...a bit of jargon but we need to make sure we use the first few fish coming from the injected parents to establish a stock... but in a month or so from now we can do an injection experiment where we flick the switch and activate TAZ-CAMTA1, and since we have colour markers, we can see if the switch is working"

The reason there is a 'switch' included that allows the TAZ-CAMTA1 to be switched on is because TAZ-CAMTA1 has proved to be highly toxic and fatal in all embryonic settings for zebrafish. The switch therefore allows the construct to be activated only when the fish is more fully developed. Ultimately the team intend to use transgenic fish with the switch and construct present so that the switch can be chemically activated.

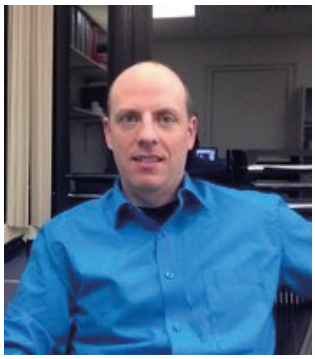
Hugh Leonard however voiced a few words of caution amongst all the excitement:

> "Assuming we are now able to create the zebrafish with a switchable EHE construct, we still don't know if that will then create the TAZ-CAMTA1 fusion protein that drives human EHE, and if we do create TAZ-CAMTA1, we don't know if it will effect endothelial cells in the same way as the human disease. However, we do have anecdotal information that zebrafish injected with the TAZ-CAMTA1 fusion protein exhibited changes to their morphology, so we remain excited by the possibilities."

We look forward to hearing more from the research team over the coming months and hope that we will have further good news to report.

Lamar Lab Updates on EHE Research

In the fall of 2021 Dr. John Lamar was the recipient of an EHE research grant jointly funded by The EHE Foundation (US) and the EHE Rare Cancer Foundation Australia to pursue research entitled TAZ-CAMTA1 Regulation by the Calcium Sensor Calmodulin.



John Lamar, PhD
Albany Medical College

In short, this research hypothesizes that targeting pathways that regulate TAZ-CAMTA1 is a promising therapeutic strategy to treat EHE and requires the

The Lamar Lab at Albany Medical College

identification of these regulatory pathways. The goal of this research is to determine if Calmodulin can repress TAZ-CAMTA1-mediated tumorigenesis (the production or formation of tumors) and to explain the mechanism by which Calmodulin regulates TAZ-CAMTA1.

This research leverages collaboration with Dr. Brian Rubin's lab using the EHE mouse cell lines generated there to perform the tests for this research. Gratefully, Dr. Lamar has added a new research technician to his team, and we are thrilled to see his lab growing. This is a multi-year research project so stay tuned.

What is Calmodulin? It is a protein which binds calcium and is involved in regulating a variety of activities in cells.

Research is Advanced by Patient's Donation to the EHE Biobank

Recently, Jenni Kovach shared with the EHE community her decision to donate lung tumors that were going to be removed during a minimally invasive surgery. Jenni contacted The EHE Foundation's Biobank Coordinator, Patty Cogswell, and provided her consent and Patty coordinated with Jenni's surgical team.

Jenni said:

> **"Patty helped make miracles happen. She has successfully worked with my hospital to ensure the tissue collection was part of the day-of-surgery plan." Jenni added, "it is so important that we get samples to the biobank. It is CRITICAL for EHE research. Please donate if you are having a procedure done. It is so easy, and the info is on the Foundation website www.fightehe.org . Here I am taking the collection box right into the operating room with me!"**

If you or a loved one with EHE has a procedure scheduled and would like to donate your tissue or fluid that needs to be drained, please contact Patty at biobank@fightehe.org. for help and learn more on how you can directly contribute to moving research forward!

The same applies in the UK and in Australia. The EHE entities in these countries can provide help and guidance also if you want to join these biobanks. Details can be found at:
UK: **www.ehercc.org.uk**
Australia: **www.ehefoundation.com.au**

02 EHE Research

A New Clinical Trial Option for Patients with EHE in the Liver

In January Jane Gutkovich shared a link to a clinical trial that may be of interest to people with hepatic EHE (EHE in the liver). Jane said:

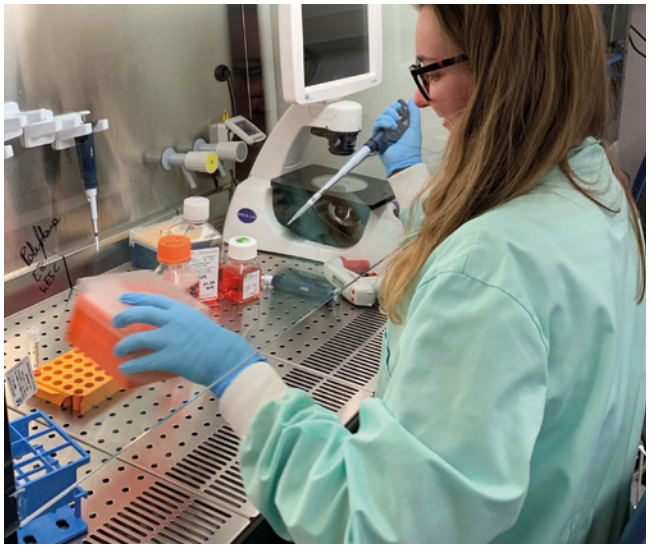
According to the device company information, the study is designed to evaluate the safety and efficacy of the company's platform technology, which uses the novel science of histotripsy, a form of therapeutic focused ultrasound to mechanically destroy targeted primary and metastatic liver tumors without invasive incisions or needles entering the patient's body.

> **"There is a clinical trial underway in several European countries (Italy, UK, Spain, Germany) and the United States which evaluates a non-invasive technology (no cutting through the skin) to destroy tumors in the liver."**

The study information and contact information can be found at **https://clinicaltrials.gov/ct2/show/NCT04573881**.

Manchester PhD continues to produce interesting results

Emily Neil completed the third year of her PhD in which she aims to develop an *in vitro* model to study the cellular and molecular characteristics of TAZ-CAMTA1 (TC) expression in endothelial cells. In prior years, Emily has developed a reliable protocol for the differentiation of mouse embryonic stem cells (mESCs) into endothelial cells, whereby TC expression can be induced with the addition of doxycycline (dox). Emily's work has continued to provide interesting results, suggesting the model she has built provides a valid platform for further investigation of the biology of EHE, and the possible evaluation of different therapeutic targets. Here is a summary of her key results over the last fifteen months.



Emily Neil, our PhD student in Manchester

1. TC expression in endothelial cells results in enrichment of TAZ target genes

RNA sequencing was used to compare the transcriptomes of TC high, TC low and TC- endothelial cells. Analysis revealed a large number of differentially expressed genes (DEGs) in cells with TC high endothelial populations (4528 DEGs), despite TC only

being induced for 24 hours. Significant enrichment in both Hippo signalling and TAZ/YAP conserved signature gene sets was also observed, as well as a significant enrichment for the EHE-specific gene set described by Seavey et al in 2021. These results suggest that the *in vitro* model developed by Emily is a relevant model to EHE.

2. TC expression in endothelial cells results in downregulation of the endothelial phenotype

Phenotypically, TC expressing endothelial cell populations show reduced tube-like formation. There was a decreased expression of multiple transcription factors involved in regulating endothelial cell phenotypes and negative enrichment scores relating to angiogenesis and maintenance of the vasculature. This suggests that TC causes transcriptional changes to genes involved in endothelial cell identity, and leads to reduced angiogenic activity.

3. TC expressing endothelial cells become arrested in S phase

As wild-type TAZ is known to regulate S phase entry in the cell cycle, and proliferation in endothelial cells, experiments were designed to determine if TC expression has a similar effect. Results showed that TC high cells were proliferating at a slower rate than TC low cells, and that TC expressing cells are indeed becoming arrested in late S phase of the cell cycle.

4. TC expression causes endothelial cells to accumulate DNA double strand breaks

One well described mechanism behind S phase arrest is the accumulation of DNA damage, as cells become arrested in S phase whilst damage is repaired to stop mutations being passed on to daughter cells. Emily was able to confirm that TC is causing substantial DNA damage involving double strand breaks (DSBs) in endothelial cells, and that this effect is seen in as little as 4 hours after TC expression is triggered. These data reveal that endothelial cells acquire a large number of DSBs upon TC expression, leading to cell cycle arrest.

5. DSBs are directly caused by TC, and are independent of its interaction with TEADs

Oncogene activation can result in DNA damage via multiple mechanisms, including inactivation of DNA damage repair pathways, production of reactive oxygen species (ROS), and replication stress. The fact that DSBs are seen just four hours after TC is induced suggests that they are directly caused by TC. In addition, this effect was also seen in cells expressing TC S51A, which cannot bind TEAD family transcription factors. These results suggest that the accumulation of DSBs is independent of TC interacting with TEAD.

The substantial change in the transcriptome 24 hours after TC expression is also suggestive of hypertranscription, an emerging source of replication stress in cancer. RNA-seq analysis and RNA imaging were used, with results suggesting hypertranscription occurs upon TC expression in endothelial cells, and could be the mechanism behind DSB accumulation.

6. Homologous recombination is impaired in TC expressing endothelial cells

Emily next investigated the ability of these cells to repair DNA damage, initially focusing on homologous recombination (HR), the DNA damage repair pathway most active in S phase of the cell cycle, where TC cells become arrested. Emily's results strongly suggest that HR is impaired in TC expressing cells. Emily has also shown that TC cells have reduced expression of BRCA1, a key protein in HR, but further experiments are needed to elucidate the mechanism behind this impairment.

7. Homologous recombination impairment leads to cellular senescence and genomic instability

It is known that HR impairment in cancer can lead to genomic instability, potentially allowing secondary mutations to occur, bypassing S phase arrest. Likewise, BRCA1-deficient cells can also become senescent (dormant), as the amount of DSBs overwhelms

compensatory repair pathways. To explore these possibilities, Emily separated and re-plated TC high, TC low and TC- cells to investigate how TC expression level affected their ability to proliferate.

Four days after sorting, the TC- population had grown to confluence, and the cells had retained their endothelial morphology. In TC high and low populations, many cells had taken on a flat morphology, characteristic of senescent cells, which was verified by the presence of the protein p16 as well as β-galactosidase activity which marks senescent cells.

Emily then moved on to determine the p16 expression level in TC-expressing endothelial cells, as this protein is known to positively regulate senescence. Moreover CDKN2A, which encodes p16, is the most common secondary mutation to TC in EHE, and these tumours are often more aggressive. Interestingly, cells with high TC expression did not express p16, however some cells with low TC levels did express p16. This suggests a transition between TC expression and p16 expression, providing further evidence that TC expression may cause oncogene induced senescence in certain situations.

Emily then generated endothelial cells which were left in culture for 4 weeks, maintaining TC expression. After 10 days, small TC+ colonies began to form, which grew in size over the course of the experiment. These colonies were not present in uninduced populations. Further testing suggested that the DSBs caused by TC expression in endothelial cells often go unrepaired, leading to cellular senescence. However a subset of cells may acquire a secondary mutation, likely due to the reduced functionality of the HR pathway. These cells do not become senescent, instead gaining a proliferative advantage as seen by the TC+ colonies. This could explain the range of disease presentation and long latency from indolent to aggressive seen in EHE.

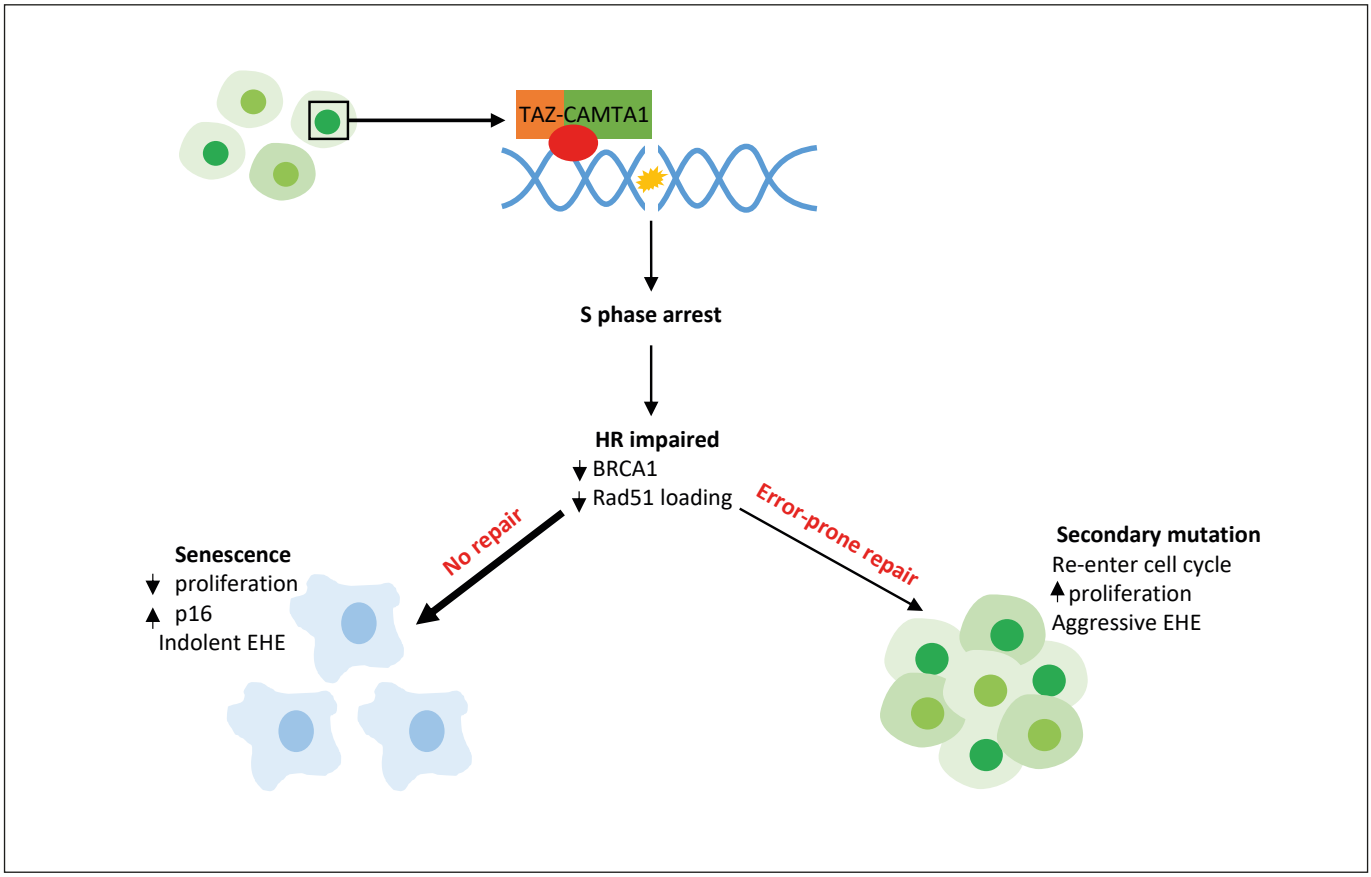
8.Knockout of CDKN2A appears to allow cells to overcome growth arrest in the cell cycle

The CDKN2A gene encodes the p16 protein, which positively regulates cellular senescence. However, CDKN2A is often found mutated in aggressive EHE tumours. Emily used CRISPR/Cas9 assays to knockout CDKN2A, and showed that in CDKN2A knockout cells expressing TC, higher growth rates were observed than in untransfected TC cells and TC increased over time, suggesting that deletion of CDKN2A allows endothelial cells to overcome the growth arrest imposed by TC expression. This is consistent with data from EHE patient samples, which shows that CDKN2A mutated EHE is more aggressive.

We want to congratulate Emily on her excellent work. As Emily's four-year PhD is now approaching its conclusion, discussions with Dr Valerie Kouskoff have started to explore how this work might be progressed, and what the key objectives of such further research should be. We look forward to reporting further on continuation of this research in future editions of The Pledge.

9.PARP inhibitors are able to induce apoptosis in TC expressing cells

Finally Emily is investigating whether PARP inhibitors are able to induce apoptosis (death) in TC expressing cells. PARP inhibitors, such as Olaparib, are commonly used to treat BRCA1 mutated cancers where impairment of the HR DNA repair pathway causes genomic instability as error-prone pathways take over. PARP inhibitors cause an increased level of DNA damage which overwhelms these compensatory repair pathways, leading to irreparable damage and apoptosis in HR deficient cells. As TC expressing endothelial cells appear to be HR deficient, they should be sensitive to PARP inhibition.



Model showing the early consequences of TC expression in endothelial cells. Upon TC expression, cells enter a state of hypertranscription which results in DSB formation. Consequently, cells become arrested in late S phase whilst DNA damage repair takes place. As HR is impaired in TC expressing endothelial cells, most will undergo oncogene induced senescence. In some cases another, more error-prone pathway will repair the DSBs and allow cells to re-enter the cell cycle. This has a high chance of causing a secondary mutation, resulting in senescence bypass and increased cell proliferation.

Emily's assay revealed that TC expressing cells were more sensitive to Olaparib than TC- cells and controls, as evidenced by a larger apoptotic population. This effect was particularly marked in the TC low populations, with TC high endothelial cells displaying a greater resistance to PARP inhibition. This may represent a mechanism by which cell death can be induced in these populations, with little effect on TC- cells.

This will require further experimentation to confirm, and Emily is currently optimising a cell viability assay, which will allow calculation of the IC50 (half-maximal inhibitory concentration) of Olaparib in these cells, as well as allowing investigation of multiple drug concentrations and drug combinations.

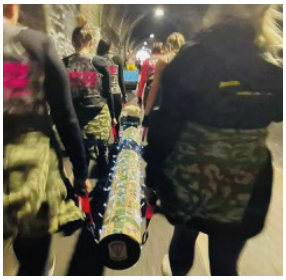
03 EHE Fundraising

Driven by the strong desire to defeat EHE, the determination of our EHE fundraisers continues to amaze and inspire us. We hope you find the following articles exciting and motivating. And remember, fundraising does not need to involve massive campaigns or extreme sports. It is often the collation of many small sums that adds up to the funding we need. If you do find the stories below inspiring, we encourage you to think about how you might be able to mobilise friends, family and colleagues to help raise the funds we need to ensure we can both maintain and expand our EHE research programme. Let's make the 2023 EHE 360 Conference even bigger!

Superwomen run half marathon for Nicola

Many people will run a half marathon to raise funds for charity, but very few will want to do that while taking it in turns to carry a 70kg (154lbs) log for the 13+ miles. But that is exactly what Nicola Henderson's friends did in late January in Yate in the UK. Nicola posted the day before the run.

> "So these are my friends... my lovely crazy friends, who tomorrow run a half marathon for our cause, not only that, they are running with a 70kg (154lb) log!! So far they have raised over £2700! They love to see your comments of support and encouragement so please leave some below! And here is the link to donate to as well."



Running from Bristol to Chipping Sodbury under their slogan "normal is boring", the 25 women known as Commando Boot Camp, trained and supported by fireman and ex-Marine Lee Ralph, smashed their initial target of £500 and raised more than a staggering £7,000 for EHE research. After the run Nicola was so grateful:

> "The team are INCREDIBLE with a legend of a leader. I can never express my gratitude to them for the amount of money and awareness raised for EHE RARE CANCER UK!"

We absolutely mirror that gratitude and praise for a really wonderful group of women and friends. Thank you ladies – you are an inspiration.

In memory of Hazel Peake

It is always with mixed emotions that we publish news of fundraising events in memory of one of our EHE Warriors who is no longer with us in person. These events can however be very special indeed as people come together to celebrate the life of a loved one and dear friend. The quiz night held in Derby in memory of Hazel Peake was just such an event.

Hazel joined the EHE group in the UK in September 2021. She quickly became a regular voice in the UK WhatsApp group and maintained a real fighting spirit as she battled her aggressive EHE. Her branch of the Unite union supported the charity with a wonderful donation of £1,000, and Hazel was excited that her colleagues were also planning a quiz night for her, and to raise funds for EHE research.

Very sadly, Hazel's health deteriorated rapidly and she passed away in late January. However, her colleagues were determined to hold their quiz night to honour and celebrate their dear friend. And what a night it was. Organised by Paul Phillips, Lisa Neal and Katie Colbourne, a huge number of Hazel's friends and colleagues were there, together with Hazel's husband Andy and their daughter Emily.

The event, held at the Rolls Royce Leisure and Event Venue in Derby raised over £10,000, thanks to the brilliant organisation and fantastic generosity of all who attended, and of all those who donated funds to the charity for EHE research. There was a lot of laughter, and just a few tears. But most of all there was a huge amount of love evident for a wonderful women.

We also want to say a huge thank you to Paul, Lisa and Katie, and to Rolls Royce who provided the facilities and the evening meal. That allowed all the funds raised on the night to go to EHE research, something we are sure Hazel would have greatly appreciated.

03 EHE Fundraising

The Americans have arrived!

Excitement was running high in London as the end of March approached, partly because the 2022 London Landmarks Half Marathon was just around the corner, but also because, for the first time since starting to participate in the run, there would be members of the EHE Foundation from America running in London.

The EHE Foundation posted the exciting news on their Facebook page on 23 March:

> "The London Landmarks Half Marathon is just a little over a week away! Please help EHE Board Member and Director of Research, Denise Robinson raise money for EHE Research. Denise is pictured on the left with her friend and running-mate, Ann, who lost her husband to EHE four years ago. Every dollar donated will go directly to research! Thank you for your support and GO DENISE & ANN!!"

Hugh Leonard explained why Denise and Ann's arrival was so exciting.

> "The amazing thing about the EHE Facebook page and our EHE community is that you make very special and deep friendships without ever actually meeting each other. So when the chance actually comes to meet people that you work with very closely, and talk to often, it really is very special. It was wonderful to meet up with both Denise and Ann on the evening of the day of their arrival, and enjoy a fun meal out in central London. There was also a lot of excitement and a little trepidation about the half marathon that was only three days away."

We also want to congratulate Denise and Ann for making the trip and we look forward to reporting on the half marathon in our next edition.

EHE RCFA launch their 2022 EHE Rare Cancer Challenge

The first quarter saw the EHE Rare Cancer Foundation Australia preparing for its inaugural national awareness and fundraising campaign during the month of April, under its banner headline:

Jonathan Granek, Founding Director of the EHE-RCFA, explained:

> "Set yourself a physical activity goal for the month of April. You can choose to walk, run, ride, swim, play, spend time at a gym or whatever your preferred physical activity is – it's up to you. Promote and obtain sponsors for your challenge and spread the word about EHE cancer and the vital need to financially support research projects."

Jonathan's first group event was tabled for the 3rd April, with a walkathon organised at the Duncan MacKinnon Reserve Walking Track. Rumour had it that free coffees might be available for those who turned up early.

We are excited about the activities, the challenges, the merchandise, and wonderful support that the EHE-RCFA always enjoys. We are looking forward to reporting about the campaign in the next edition of The Pledge.

03 EHE Fundraising

EHE Fun Run and Walk teams up with Maine Marathon

Kim Young was excited to share news of her Maine Marathon plans.

> “It's 2022 and as everyone sets new goals for themselves this year, I'm excited to announce that The EHE Foundation has been added as a charity bib partner for the Maine Marathon! This means that if you register for any event, virtual or in-person, you can raise money for the foundation through fundraising efforts. Donations can easily be collected directly through your unique fundraising website provided by the Maine Marathon.”

Kim is teaming up with The EHE Foundation for their fall EHE Fun Run and Walk. She plans to run the full marathon again this year and she's looking for anyone who might want to run any distance with her and help raise funds to fight EHE. Whether you want to run your first full marathon or walk a 5k around your neighborhood, please consider signing up!
You can even run/walk the 2022 EHE Fun Run and log it toward your virtual 5k for the Maine Marathon! Double whammy!

Here's the link to register for the marathon (only) **https://runsignup.com/Race/ME/Portland/MaineMarathon.**

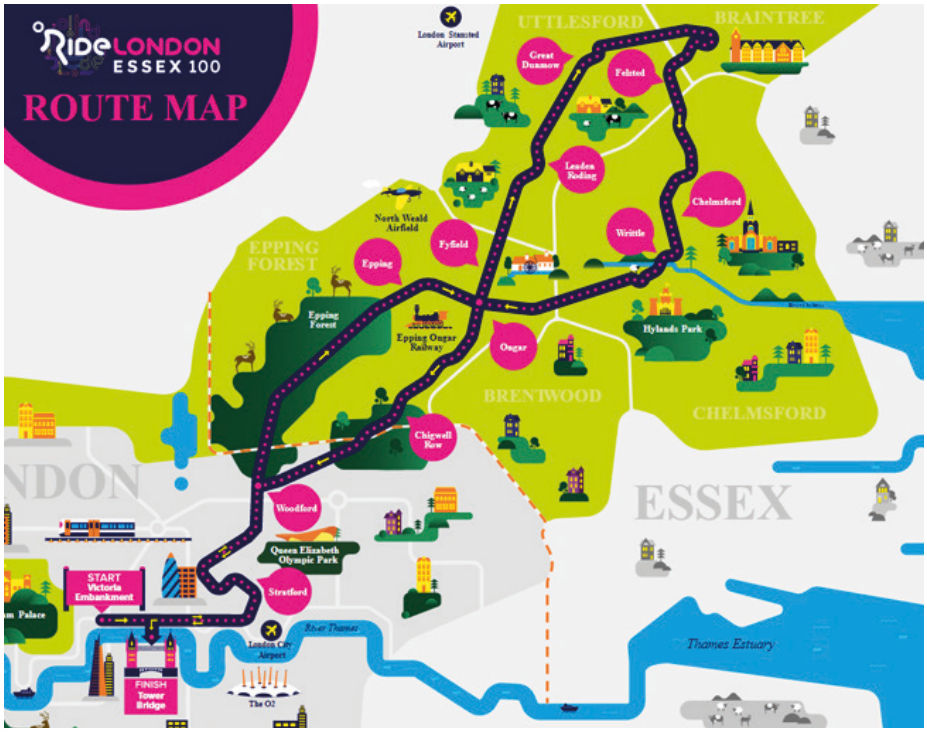
Stay tuned for more information regarding The EHE Foundation's 2022 Fun Run and Walk set for this fall.

Ride London 2022 is on and EHERCC riders are taking part!

The Ride London 100 was a regular event in the EHE Rare Cancer Charity's fundraising calendar. However the 2020 event was cancelled due to COVID and 2021 was lost when Surrey decided not to support the event south of London. But in late 2021 the organisers announced that the ride would be moving to north of London and was back on. EHERCC moved to immediately secure places, confirmed by the EHERCC in January:

> "We have secured 20 places in the NEW RideLondon100 cycling event which will be on 29 May. This 100 mile closed-roads event will this year start in central London, then go out into Essex, and then return and finish at the iconic London Bridge. So please come and join us as we ***Just Live*** and Just Ride too!"

The Charity has already filled all its 20 places, with participants now training hard for the big day. Hugh Leonard, who is part of the team said: "its both very exciting but also quite daunting for those of us who do not ride regularly, but we are up for the challenge!!". We wish all the riders good luck and thank them for their dedication.



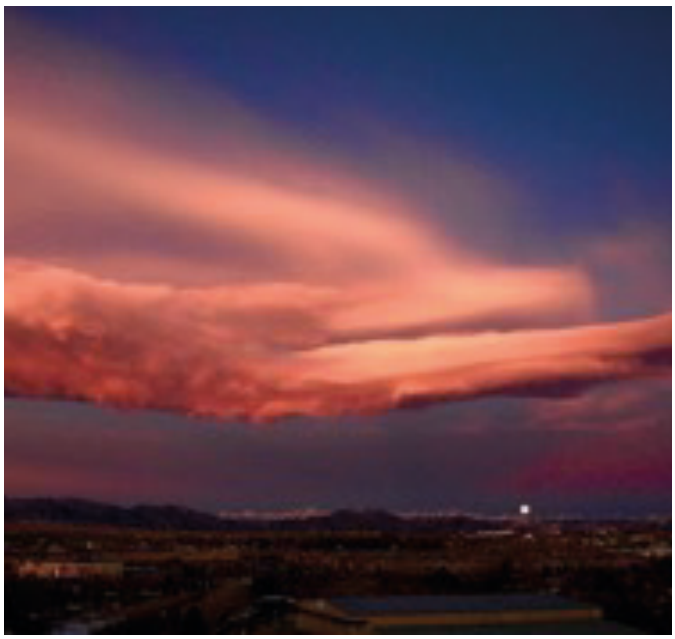
04 And in other news...

Every quarter, people will post stories or short messages that are not necessarily related to EHE. We always include a small selection of these as we think it supports the spirit of so many of our EHE community, namely that they will not let EHE control or dictate how they lead their lives. They will ***"Just Live!"*** Here are the posts for this quarter.

The photo-therapy section

Here are this quarters photographs, posted by Carl Dickson.

> "My symptoms have not been letting me get much sleep lately but the benefits and the reward that the beauty of Colorado nights and early, early mornings can bring to the eyes and soul outweigh the downside. Hope those having difficulty getting out right now might enjoy these two photos."



Getting together:

We always love photos of our EHE family members meeting up and getting together. This one is from London where Hugh Leonard and Sally Baker were delighted to have the chance to visit the iconic Hampton Court Palace with Denise Robinson and Ann Campbell the afternoon before the London Landmarks Half Marathon.

The EHE Foundation (USA)
www.fightehe.org

The EHE Rare Cancer Charity (UK)
www.ehercc.org.uk

The EHE Rare Cancer Foundation (Australia)
www.ehefoundation.com.au

EHE Italia - Non solo Laura
website not yet available

EHE Canada
website not yet available