International Scientific Meeting for PIK3CA Related Conditions

October 28-29, 2021

Scientific Organizing Committee

Denise Adams, MD, Children's Hospital Philadelphia, Philadelphia, PA, USA
Miikka Vikkula,MD, Ph.D., de Duve Institute, Brussels, BE
Jean Zhao, Ph.D., Dana-Farber Cancer Institute, Boston, MA, USA

The planning committee for this meeting includes the following patient advocacy organizations:

CLOVES SYNDROME COMMUNITY

M-CM NETWORK

"What do we live for, if not to make life less difficult for each other." – George Eliot –

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Conference Agenda - All times are in Eastern Standard Time

Thursday, October 28, 2021

10:00a-11:05 EST **Opening Session**

10:00 **Welcome Address: Kristen Davis**, Organizing Chair & Executive Director CLOVES Syndrome Community

10:10 Keynote Patient Journey Video

10:20 **Keynote Address: Lewis Cantley, PhD** The Importance of Collaboration for the Future of Clinical Research and Treatments *(Eligible for CME credit)*

11:05-12:40p **Session 1: Basic Science**

11:05 **Introduction**-Guillaume Canaud, MD, PhD *(Eligible for CME credit)*
Session Chair: Jean Zhao PhD

11:50 **Johanna Laakkonen PhD** *Missense Mutations in PIK3CA gene in Angiomatosis of Soft Tissue*

12:00 **Catherine Cottrell PhD** *Use of Paired Exome Analysis in Vascular Anomalies Expands the Associated Genetic Spectrum*

12:10 **Astrid Eijkelenboom PhD** *Sharing experiences: five years of NGS based somatic PIK3CA variant detection on FFPE tissues in routine diagnostics for vascular malformations*

12:20 **Chiara Borsari PhD** *Volume Scanning, a Rational Approach to Covalent PI3Kα Inhibitors*

12:30 **Session 1: Basic Science Q&A**

12:40-1:10 **Break**

1:10 **Patient & Caregiver Video: The Diagnostic Journey**

1:20-3:15 **Session 2: Diagnosis and Natural History**

1:20 **Introduction**-Session Chair: Denise Adams, MD *(Eligible for CME credit)*

2:05 **Pascal Brouillard PhD** *Non-Hotspot PIK3CA Mutations are More Frequent in CLOVES than in Common or Combined Lymphatic Malformations.*

2:15 **Sofia Douzgou Houge MD, PhD** *A standard of care for individuals with PIK3CA-related disorders: an international expert consensus statement*

2:25 **Kim Keppler-Noreuil MD** *Characterization and Tumor Risk in PIK3CA-related overgrowth spectrum (PROS)*

2:35 **Nagore Gene PhD** *ddPCR: a novel technique for molecular diagnosis of vascular anomalies*

2:45 **James Bennett MD, PhD** *Non-invasive diagnosis of PIK3CA mutations in individuals with lymphatic malformations using cyst-fluid derived cell free DNA*

2:55 **Paloma Triana MD** *Lower limb overgrowth associated with first toe undergrowth in PIK3CA patients*

3:05 **Session 2: Diagnosis and Natural History Q&A**

3:15 **Networking/Social Event**

Friday, October 29, 2021

9:30am EST **Sponsor Symposium: Venthera**

10:00 **Welcome Address: Mandy Sellars,** Founder and Chairperson of GoPI3Ks

10:10 **Patient & Caregiver Video: Day to Day Life**

10:20-11:35 **Session 3: Pre-clinical Research**

- 10:20 **Introduction**-Session Chair: Miikka Vikkula MD, PhD *(Eligible for CME credit)* Session Co-Chair: Elisa Boscolo PhD
- 11:05 **Achira Roy PhD** *Determining underlying mechanisms and preclinical treatment for PIK3CA-driven hydrocephalus and epilepsy*
- 11:15 **Matthias Wymann PhD** *Novel, highly potent PI3Kalfa covalent inhibitors deconvolute class I PI3K isoform signaling*
- 11:25 **Session 3: Pre-clinical Research Q&A**

11:35-12:05p **Break**

12:05 **Patient & Caregiver Video: The Impact of Treatment**

12:15-2:00 **Session 4: Treatments and Innovation**

- 12:15 **Introduction**-Session Chair: Adrienne Hammill MD, PhD *(Eligible for CME credit)*
- 1:00 **An Van Damme MD, PhD** *Sirolimus for in utero management of large fetal LM*
- 1:10 **Abhay Srinivasan MD** *Central conducting channel anomaly with associated PI3KCA variant presenting with rectovaginal leak*
- 1:20 **Emmanuel Seront MD, PhD** *Preliminary results of VASE trial evaluating Sirolimus in Vascular Malformations refractory to Standard Care: subgroup analysis of PIK3CA-mutated patients.*
- 1:30 **Satyamaanasa Polubothu PhD** *Post-zygotic mutations in NRAS and PIK3CA in a case series of generalised lymphatic anomaly (lymphangiomatosis)*
- 1:40 **Ananya Majumdar PhD** *Procedural Treatment Outcomes for Fibro-Adipose Vascular Anomaly*
- 1:50 **Iryna Benzar MD** *Lateral marginal vein in children with CLOVES and Klippel-Trénaunay syndromes: is early treatment the best choice?*
- 2:00 **Session 4: Treatments and Innovation Q&A**

2:10 **Break**

2:20 **Challenges and Opportunities in Research: A Panel Discussion** Moderators: Dr. Miikka Vikkula MD, PhD and Dr. Jean Zhao PhD with Dr. Sarah Sheppard MD, PhD, Dr. Ralitsa Madsen, PhD, Dr. Friedrich Kapp and Dr. Timothy Le Cras, PhD

2:55 **Closing Remarks**

Non-invasive diagnosis of PIK3CA mutations in individuals with lymphatic malformations using cyst-fluid derived cell free DNA

James Bennett[1]

[1]*Seattle Childrens Hospital*

Introduction: Vascular malformations (VM) are primarily caused by somatic activating pathogenic variants in oncogenes, including PIK3CA which is the primary genetic cause of isolated lymphatic malformations. Targeted pharmacotherapies are emerging but require molecular diagnosis. Since variants are currently only detected in malformation tissue, patients may be ineligible for clinical trials prior to surgery. We hypothesized that cell-free DNA (cfDNA) could provide molecular diagnoses for patients with isolated lymphatic malformations.

Methods: cfDNA was isolated from plasma or cyst fluid from patients with lymphatic malformations (LM), and assayed for known pathogenic variants using droplet digital polymerase chain reaction (ddPCR). Cyst fluid cfDNA from an independent cohort of LM patients was prospectively screened for variants using a multiplex ddPCR assay.

Results: PIK3CA variants were detected in cyst fluid cfDNA (7/7) but not plasma (0/26) in LM patients. Prospective testing of cyst fluid cfDNA with multiplex PIK3CA ddPCR identified variants in LM patients who had never undergone surgery (4/5).

Conclusion: Variants were detected in in cyst fluid, but not in plasma from patients with PIK3CA related LM. These data support investigation of cfDNA-based molecular diagnostics for PROS related conditions, which may provide opportunities to initiate targeted pharmacotherapies without prior surgery.

Lateral marginal vein in children with CLOVES and Klippel-Trénaunay syndromes: is early treatment the best choice?

Iryna Benzar[1] & Borys Koval[1]

[1]*Bogomolets National Medical University*

Objectives. We evaluated the effectiveness of early marginal vein endovascular interventions aimed to reduce the symptoms and prevent thromboembolic complications in children.

Methods. 4 children were enrolled with persistent marginal vein, 2 boys and two girls, aged 3-12 years. The marginal vein vas right sided in 2 children, left sided in 1 and both legs affected in 1. According to Weber classification, among 5 marginal veins there were type 3 (n=1), type 4 (n=3), and type 5 (n=1). One child had CLOVES syndrome, and 3 children had Klippel-Trénaunay syndrome. All children required multiple surgical interventions for vascular and osseous malformations.

Results. Sonography and MRI angiography confirmed the diagnosis. Endovenous laser coagulation was performed in all cases. In 1 patient, coil embolization of proximal marginal vein part caused its progressive expansion due to the high pressure bloodflow through the tributaries with the common femoral vein. Preprocedural LWH therapy was administered in patient with coagulopathy. Tumescence was performed with cold normal saline. The guide wire was inserted maximally to the distal part of vein. Endovascular laser coagulation was performed with laser energy at 8-10 W, 80-100 J/cm in the segment from 25 to 40 cm long. All procedures were performed under general anesthesia without complications. 3-8 months follow up showed occlusion of the laser-treated venous parts, limb circumference reduction, and skin lesions improvement.

Conclusions. Endovascular laser ablation of marginal vein is safe and effective method of treatment in young children and toddlers and should be performed before following surgical interventions.

Volume Scanning, a Rational Approach to Covalent PI3Kα Inhibitors

Chiara Borsari[1] & Matthias Wymann[1]

[1]*University of Basel*

Fibro-Adipose Vascular Anomaly (FAVA) is an overgrowth condition that stems from a PIK3CA mutation. FAVA is characterized by a dense fibrofatty infiltration of muscle tissue that manifests with mass, phlebectasia, pain and loss of function.

Objectives:
Compare surgical resection, sclerotherapy, and cryoablation, to evaluate FAVA treatment outcomes and postoperative quality of life.

Methods:
A retrospective chart review was conducted on a group of 22 FAVA patients. Preoperative and postoperative information regarding the following variables were collected: lesion size, pain presence, pain intensity, medications, flares, pain management, ED admissions, sleep disturbance, ability to play sports, and effects on daily life.

Results:
For outcomes, cryoablation (t-test 8.52, significance 0.002), sclerotherapy (t test 3.99, significance 0.002), and surgery (t-test 17, significance 0.037) reduced postoperative pain intensity. Cryoablation and surgery had no lesion recurrences within 482 and 97.5 days respectively, whereas sclerotherapy had an average of 0.71 recurrences per patient within 242 days. Furthermore, sclerotherapy decreased lesion size by 13.7%, whereas cryoablation and surgery decreased by 92.75% and 100% respectively. For quality of life, 50% of surgery patients missed school due to pain, and 50% of surgery patients and 37.5% of sclerotherapy patients were unable to play sports. Sclerotherapy patients reported flares in 25% of patients and 3 instances of pain management related ED admissions. Cryoablation patients reported no such postoperative, pain related ED admissions.

Conclusions:
Cryoablation and surgery were the best procedural treatments for reducing lesion size and pain, while cryoablation demonstrated the best postoperative quality of life in the follow-up period.

Non-Hotspot PIK3CA Mutations are More Frequent in CLOVES than in Common or Combined Lymphatic Malformations.

Pascal Brouillard PhD [1] , Matthieu J. Schlögel, PhD,[1], Nassim Homayun Sepehr, MSc,[1] , Raphaël Helaers, PhD,[1] , Angela Queisser, PhD,[1] , Elodie Fastré, PhD,[1] , Simon Boutry, MSc,[1] , Sandra Schmitz, MD, PhD[2,3,5] , Philippe Clapuyt, MD[6,3,5], Frank Hammer, MD, [6,3,5], Anne Dompmartin, MD, PhD[7] , Annamaria Weitz-Tuoretmaa, MD[8] , Jussi Laranne, MD, [9], Louise Pasquesoone, MD,10 , Catheline Vilain, MD, [11], Laurence M. Boon, MD, PhD[1,3,5], & , Miikka Vikkula, MD, PhD[1,3,5,12]

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[12] Walloon Excellence in Lifesciences and Biotechnology (WELBIO)

Background:
Theragnostic management requests to unravel patients' genotypes. We screened for somatic PIK3CA mutations on lesional tissue or lymphatic endothelial cells isolated from lesions. Our cohort (n=143) included patients with a common lymphatic malformation (LM), a combined lymphatic malformation [lymphatico-venous malformation (LVM), capillaro-lymphatic malformation (CLM), capillaro-lymphatico-venous malformation (CLVM)], or a syndrome [CLVM with hypertrophy (Klippel-Trenaunay-Weber syndrome, KTS), congenital lipomatous overgrowth-vascular malformations-epidermal nevi -syndrome (CLOVES), unclassified PIK3CA-related overgrowth syndrome (PROS) or unclassified vascular (lymphatic) anomaly syndrome (UVA)].

Results:
We identified a somatic PIK3CA mutation in 108 out of 143 patients (75.5%). The variant allele frequency (VAF) ranged from 0.54% to 25.33% in tissues, and up to 47% in isolated endothelial cells. We detected a statistically significant difference in the distribution of mutations between patients with common and combined LM compared to the syndromes, but not with KTS. Moreover, the VAF was higher in the syndromes.

Conclusions:
Most patients harbour a somatic PIK3CA mutation. However, in about a quarter, no mutation was detected, suggesting the existence of (an)other cause(s). We detected hotspot mutations more frequently in common and combined LMs than in syndromic cases (CLOVES and PROS). Diagnostic genotyping should thus not be limited to PIK3CA hotspot mutations. Moreover, the higher VAF in syndromes suggests a wider distribution in patients' tissues, facilitating detection.
Clinical trials have demonstrated efficacy of Sirolimus and Alpelisib in treating patients with an LM or PROS. Genotyping might lead to an increase in efficacy, as responses could vary depending on presence and type of PIK3CA-mutation.

Use of Paired Exome Analysis in Vascular Anomalies Expands the Associated Genetic Spectrum

Catherine E. Cottrell[1,2], Bhuvana A. Setty[3,4], Anna P. Lillis[5,6], Ibrahim Khansa[7], Gregory D. Pearson[7], Esteban Fernandez Faith[4,8], Patricia M. Witman[4,8], Bayan A. Matarneh[4,8], Katya L. Harfmann[4,8], Amanda Whitaker[4,9], Richard E. Kirschner[7], Kim A. Bjorklund[7], Jonathan M. Grischkan[10], Kris R. Jatana[10], Patrick C. Walz[10], Meredith N. Lind[10], Leah Braswell[5], Sara Smith[5], Raimie Lewis[5], Mai-Lan Ho[5,6], Jordan Halsey[7], Archana Shenoy[2,11], Jennifer H. Aldrink[12] , Mari Mori[4,13], Andrea A. Wojtowicz[14], Amanda E. Jacobson-Kelly[3,4], Aimee Jalkanen[1], Mariam Mathew[1,2,] Kristy Lee[1,2], Elizabeth Varga[1], Samantha Choi[1], Bhoomi Patel[1], Kristen Leraas[1], Patrick Brennan[1], Benjamin Kelly[1], Kathleen M. Schieffer[1], Peter White[1,4], Vincent Magrini[1,4], Richard K. Wilson[1,4], Elaine R. Mardis[1,4]

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Objectives: The classification of vascular anomalies (VA) can be challenging, requiring expert clinician engagement, as well as diagnostic studies to further aid in characterization. ISSVA provides a framework for classification, however the genetic contributors to disease and variant spectrum are yet evolving. Sensitive molecular techniques have enabled advances in our understanding of the genetic etiology of vascular malformations, vascular tumors, and somatic disease. Current approaches to genomic profiling are variable in scope. Herein, we examine the impact of comprehensive analysis of paired tissue sources by exome sequencing.

Methods: Within our pediatric tertiary care institution, a multidisciplinary team of expert clinicians delivers high-quality patient care for individuals with VA. Among a cohort of 22 individuals with vascular malformations (n=17) or vascular tumors (n=5), genomic studies included paired somatic disease-germline comparator exome analysis targeted at 250X.

Results: The diagnostic yield within this cohort was 68%, with 3 germline and 13 somatic disease-associated genetic variants identified among 15 of 22 individuals. Variants occurred primarily within the PI3K-AKT and RAS-MAPK pathways. Outside of oncogenic hotspots, which comprised 54% of detected somatic variants (n=7; PIK3CA(4), MAP2K1(2), GNAQ(1)), insertion-deletions (n=6; HRAS(1), MAP2K1(1), MAP3K3(1), PTCH1(2), TIE1(1)) were enriched in well-described and candidate genes.

Conclusion: Paired exome sequencing is a sensitive method to capture germline and somatic variation among known and emerging gene targets. PIK3CA was the most frequently altered gene in this VA cohort. Insertion-deletions represented nearly half of disease-associated somatic variants. Knowledge of the genetic etiology of VA can aid in diagnosis, management, and therapeutic consideration.

A standard of care for individuals with PIK3CA-related disorders: an international expert consensus statement

Sofia Douzgou,[1,2,3*] Myfanwy Rawson,[2] Eulalia Baselga,[4] Moise Danielpour,[5] Laurence Faivre,[6*] Alon Kashanian,[5] Kim M Keppler-Noreuil,[7] Paul Kuentz,[8] Grazia MS Mancini,[9] Marie-Cecile Maniere,[10] Victor Martinez-Glez,[11,12,13] Victoria E Parker,[14] Robert K Semple,[15] Siddharth Srivastava,[16] Pierre Vabres,[6] Marie-Claire Y de Wit,[17] John M Graham Jr,[18] Jill Clayton-Smith,[2,3] Ghayda M Mirzaa[19] and Leslie G Biesecker[20]

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* Member of the European Reference Network ERN-ITHACA

Objectives
Growth promoting variants in PIK3CA cause a spectrum of developmental disorders, depending on the developmental timing of the mutation and tissues involved. These entities have been grouped as PIK3CA-Related Overgrowth Spectrum disorders (PROS). Deep sequencing technologies have facilitated detection of low-level mosaicism, often necessitating testing of tissues other than blood. Since clinical management practices vary considerably among healthcare professionals and services across different countries, a consensus on management guidelines is needed. Clinical heterogeneity within this spectrum leads to challenges in establishing management recommendations, which must be based on patient-specific considerations. Moreover, as most of these conditions are rare, affected families may lack access to the medical expertise that is needed to help address the multi-system and often complex medical issues seen.

Methods
In March 2019, macrocephaly-capillary malformation (M-CM) patient organizations hosted an expert meeting in Manchester, United Kingdom, to help address these challenges with regards to M-CM syndrome. We expanded the scope of this project to cover PROS. The process included literature curation, agreeing and scoring recommendations using the AGREE II Tool and a 2-year-long electronic correspondence among the expert group to agree the final version of the document.

Results
We developed a consensus statement on the preferred approach for managing affected individuals based on our current knowledge.

Conclusions
We believe that outlining clinical management guidelines is essential in view of the on-going clinical trials and up-and-coming treatments for PROS. A standard of care can be useful for monitoring the effects of such trials or caring for non-responders.

Sharing experiences: five years of NGS based somatic PIK3CA variant detection on FFPE tissues in routine diagnostics for vascular malformations

Astrid Eijkelenboom[1], Carine van der Vleuten[1], & Uta Flucke[1]

[1]*Radboudumc*

OBJECTIVES: Vascular malformations encompass a spectrum of lesions of which clinical and pathological classification can be challenging. These lesions are characterized by somatic/mosaic pathogenic variants and consequently result in variable clinicopathological presentation. Clinical management of the lesions can be challenging and requires multidisciplinary evaluation in expertise centers. As one of few in Europe, we provide routine Next Generation Sequencing based molecular diagnostic tissue analysis since 2017. Here, we provide an overview of our routine diagnostic analyses with key findings.
METHODS: Molecular screening of regions in PIK3CA and 17 other genes using targeted NGS with Unique Molecular Identifiers and a lower level of detection of 1% mutant allele was performed on 823 clinical paraffin-embedded formalin-fixed tissuesections.

RESULTS:
Overall, in 323 cases pathogenic variants were identified, of which 178 'PIK3CA-mutated' cases, showing that:
I) Three 'hotspot mutations' (p.E542K, p.E545K and p.H1047R) together represent the vast majority of identified pathogenic variants in PIK3CA (84%).
II) Pathogenic variants are identified with a low mutant allele frequency (77% of variants were present at a mutant allele frequency <10%), urging the use of sensitive detection-methods and stringent quality-control such as binomial distribution based minimal coverage thresholds.
III). Ten cases surprisingly harbored, not previously described, combinations of known PIK3CA and TEK, GNA11, GNA14, GNAQ or IDH1 pathogenic variants.

CONCLUSION: Sensitive NGS-based screening in vascular malformations is required to properly evaluate PIK3CA-status for diagnosis and potential therapeutic options. The co-existence of two activating mutations in parallel pathways illustrates potential treatment challenges and underlines the importance of multigene testing.

ddPCR: a novel technique for molecular diagnosis of vascular anomalies

Nagore Gene[1] & Cinzia Lavarino[1]

[1] *Hospital Sant Joan de Déu*

There is a strong need for accurate and sensitive methodologies for precise molecular diagnosis of vascular anomalies, and thus identify patients that can benefit from specific targeted therapy. We have implemented a highly precise approach for sensitive and reproducible detection and quantification of DNA variants using droplet digital PCR (ddPCR). ddPCR enables accurate quantification of the absolute number of low-abundance DNA molecules present in patient-derived samples, including small biopsies, circulating tumor DNA in plasma and lymphatic fluid. We have employed and validated commercial as well as custom designed ddPCR assays for the detection of PIK3CA, TEK, GNA11 and GNAQ hotspot mutations with extremely low variant allele frequency (VAF) variants (VAF <0.5%). We have analyzed more than fifty fresh-frozen and formalin-fixed paraffin embedded (FFPE) samples of vascular anomalies and identified an oncogenic mutation in more than sixty per cent of the samples with a VAF median of 5% (range 0.5-13.5%). We are currently optimizing the use of ddPCR for DNA detection in liquid biopsies such as plasma and lymphatic fluid to enable the molecular characterization of vascular anomalies using a minimally invasive, rapid method.
Based on our experience, we purpose ddPCR as a clinically applicable approach for accurate, rapid and cost effective detection of specific DNA variants for molecular diagnosis of vascular anomalies and potential treatment target detection.

Characterization and Tumor Risk in PIK3CA-related overgrowth spectrum (PROS)

Kim Keppler-Noreuil[1]

[1]*University of Wisconsin -Madison School of Medicine and Public Health, Dept of Pediatrics*

Objectives: Genetic and epigenetic syndromes associated with lateralized overgrowth have been associated with increased risk of primarily embryonal tumors, including Wilms tumor (WT), with frequency of tumor development between 3.3 -6%. The PI3K-AKT-mTOR comprise a critical signaling pathway regulating cellular growth, proliferation, and angiogenesis. Many different solid and hematological tumors or cancers are caused most commonly by somatic activating variants in PIK3CA hotspot codons H1047, E542, and E545. Tumor risk and surveillance for patients with PROS is currently controversial. The objectives of this systematic review are to characterize and determine the estimated risk for tumorigenesis and development of malignancies in PROS.

Methods: Retrospective review of the literature for PROS phenotypes and reported tumors/malignancies.

Results: There have been 11 reports of PROS in the literature, including 12 individuals, who have developed tumors. Frequency ranged from 1.4% (6/419), 1.6% (4/258) to 3.3% (4/122). Clinical diagnoses included 6 with CLOVES, 2 with MCAP, 2 with KTS, 2 with lateralized overgrowth. The tumor types included: 7 (~60%) with WT, 4 (33%) with indeterminate WT vs Nephroblastomatosis (NB), and 1 (8%) with NB. Six (50%) had somatic PIK3CA hotspot variants. Age at tumor diagnosis was 27.4 months (mean), and 9-119 months (range). Urine cell-free DNA detected low level PIK3CA in these reported patients with NB or WT compared to those without renal involvement ($P < 0.05$).

Conclusions: Proposed surveillance and clinical management will be reviewed. Further longitudinal data is needed to support relationship between tumor risk and genotype/ clinical phenotypes to guide recommendations for surveillance.

Missense Mutations in PIK3CA Gene and Intervascular Stromal Cells in Angiomatosis of Soft Tissue

Johanna Laakkonen[1]

[1]*A.I. Virtanen Institute*

Background. Angiomatosis of soft tissue (AST) is a benign intramuscular vascular anomaly affecting venous vasculature. The symptoms often include pain and functional impairment. Histologically AST consists of vessels of different origin and size, of which most prominent are wide venous channels and artery-like vessels. Muscle-infiltrating fat is also abundant.

Objectives. AST can be misdiagnosed as intramuscular, common venous malformation (VM). Differential diagnosis of these two entities is important, as sclerotherapy is suitable for most VMs but is inefficient in AST. Methods. We characterized somatic mutations in 31 non-skin associated venous lesions, of which 20 were AST and 11 VM. Patient-derived cells were used to understand the crosstalk of endothelial cells and intervascular stromal cells in venous lesions.

Results. In AST, 16 out of 20 patients carried a missense mutation in PIK3CA gene, whereas only 1 out of 11 patients with VM had a PIK3CA mutation. Pathogenic PIK3CA variants were accordingly found in AST-derived endothelial cell lines. AST-derived intervascular stromal cells were shown to secrete growth factors, and to induce a pro-angiogenic phenotype of genotypically normal endothelial cells.

Conclusions. AST patients having extensive or infiltrating lesions with a missense mutation in PIK3CA gene could benefit from treatment with PI3K/AKT/mTOR inhibitors. Intervascular stromal cells induce lesion formation in AST.

Procedural Treatment Outcomes for Fibro-Adipose Vascular Anomaly

Craig Johnson[1] & **Ananya Majumdar[1]**

[1]*University of Central Florida College of Medicine- Nemours Children's Hospital*

Fibro-Adipose Vascular Anomaly (FAVA) is an overgrowth condition that stems from a PIK3CA mutation. FAVA is characterized by a dense fibrofatty infiltration of muscle tissue that manifests with mass, phlebectasia, pain and loss of function.

Objectives:
Compare surgical resection, sclerotherapy, and cryoablation, to evaluate FAVA treatment outcomes and postoperative quality of life.

Methods:
A retrospective chart review was conducted on a group of 22 FAVA patients. Preoperative and postoperative information regarding the following variables were collected: lesion size, pain presence, pain intensity, medications, flares, pain management, ED admissions, sleep disturbance, ability to play sports, and effects on daily life.

Results:
For outcomes, cryoablation (t-test 8.52, significance 0.002), sclerotherapy (t test 3.99, significance 0.002), and surgery (t-test 17, significance 0.037) reduced postoperative pain intensity. Cryoablation and surgery had no lesion recurrences within 482 and 97.5 days respectively, whereas sclerotherapy had an average of 0.71 recurrences per patient within 242 days. Furthermore, sclerotherapy decreased lesion size by 13.7%, whereas cryoablation and surgery decreased by 92.75% and 100% respectively. For quality of life, 50% of surgery patients missed school due to pain, and 50% of surgery patients and 37.5% of sclerotherapy patients were unable to play sports. Sclerotherapy patients reported flares in 25% of patients and 3 instances of pain management related ED admissions. Cryoablation patients reported no such postoperative, pain related ED admissions.

Conclusions:
Cryoablation and surgery were the best procedural treatments for reducing lesion size and pain, while cryoablation demonstrated the best postoperative quality of life in the follow-up period.

Post-zygotic mutations in NRAS and PIK3CA in a case series of generalised lymphatic anomaly (lymphangiomatosis)

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Generalised lymphatic anomaly (GLA) is a rare, sporadic, congenital disorder characterised by diffuse or multicentric proliferation of lymphatic vessels, leading to disruption of normal tissue architecture and organ dysfunction. Treatment is challenging due to the progressive and infiltrative nature of the disease. Sclerotherapy and surgical debulking can offer symptomatic treatment but currently there is no cure. Somatic mutations in the genes PIK3CA and NRAS have been recently described in GLA which raises the possibility of targeted medical therapies for these cohorts. The aim of this study was to investigate the genetic cause of GLA in a series of patients, with a view to rationalising treatment strategies.

Ten patients with GLA were recruited. DNA was extracted directly from affected tissue and sequenced on a high-depth targeted next generation sequencing panel (SureSeq Solid Tumour Panel, Oxford Gene Technology). Known pathogenic variants in genes NRAS (n=2), both c.182A>G, p.(Q61R), and PIK3CA (n=4), c.1633G>A; PIK3CA p.(E545K) (n=1), c.3140A>G; PIK3CA p.(H1047R) (n=2) and c.1624G>A; PIK3CA p.(E542K) (n=1), were detected in affected tissue, allele load ranging between 1-23%. Therapy with the mTOR inhibitor Sirolimus (thus downstream of PIK3CA and NRAS) was used prospectively in six patients leading to improvement in three patients and stabilisation in a further three.

In conclusion, we describe somatic variants in PIK3CA and NRAS in a series of children with GLA. With the advent of the use of targeted inhibitors of PIK3CA and MEK in complex vascular anomalies, routine genotyping of GLA will allow stratification for personalised targeted therapies.

Determining underlying mechanisms and preclinical treatment for PIK3CA-driven hydrocephalus and epilepsy

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Objective: Activating mutations in PIK3CA, long studied for roles in cancer, also cause clinically important developmental brain overgrowth syndromes. Affected individuals display a range of malformations including cortical dysplasia, developmental hydrocephalus, epilepsy and intellectual disability. A significant proportion of these patients remain unresponsive to conventional medications/treatments. Understanding mutation-specific pathophysiology is thus critical for developing molecularly targeted therapies.

Methods: We previously generated genetic mouse models of human–related Pik3ca mutations to recapitulate the key pathological features. Using these models and different cre lines, we sought to determine mechanisms underlying Pik3ca-related hydrocephalus and epilepsy. Further, specific pathway inhibitors were administered to preclinically study their potential therapeutic actions on these anomalies.

Results: We identified that activating Pik3ca mutations has effect on the size of brain or lateral ventricles only when acquired during embryogenesis; however, Pik3ca mutants remain epileptic irrespective of time of mutation induction. We determined that dysregulation in PI3K-Yap pathway interactions led to stereotypic cortical gyrification and ventriculomegaly in the mutants, that lead to hydrocephalus. Intriguingly, administration of Yap inhibitor attenuated these phenotypes by controlling mutation-related defects in cell-cell adhesion and proliferation.
Parallelly, we report that Pik3ca-driven epileptiform hyperexcitability is mediated by changes in multiple non-synaptic, cell-intrinsic properties. By repurposing PI3K pathway inhibitors originally developed to treat cancer, we unravel that acute inhibition of PI3K or AKT, but not MTOR, suppresses the Pik3ca-driven intrinsic neuronal hyperactivity.

Conclusion: Our data demonstrate a distinct mechanistic milieu underlying Pik3ca-driven developmental hydrocephalus and intractable epilepsy, and promise potential precision therapeutics towards their treatment.

Preliminary results of VASE trial evaluating Sirolimus in Vascular Malformations refractory to Standard Care: subgroup analysis of PIK3CA-mutated patients.

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Objectives
To analyse efficacy of sirolimus in PIK3CA-mutated patients with slow-flow vascular malformations.

Methods
The preliminary results of the prospective ongoing VASE trial reported an efficacy of sirolimus in 82% of patients with symptomatic slow-flow vascular malformations. In this trial, sirolimus is stopped after 2 years of treatment, but can be reintroduced in case of symptoms resurgence. Outcome of PIK3CA-mutated patients is reported.

Results
At March 2021, among the 112 patients with a follow-up of at least 6 months after the onset of sirolimus, 20 had a PIK3CA mutation (10 venous malformations (VM), 2 capillaro-venous malformations (CVM), 2 lymphatic malformations (LM), 2 capillaro-lymphatico-venous malformations (CLVM), 1 capillary malformation with dilated veins (CMDV), 2 CLOVES and 1 Klippel-Trenaunay syndrome.
Out of these 20 PIK3CA-mutated patients, 17 patients (85%) reached a ≥75%-improvement in symptoms with a median time to reach this response of 2 months (range 1-6months). Twelve patients completed the 2-year treatment; 4 had to restart sirolimus within the first 3 months after arrest and 1 after 9 months. In comparison, among the 17 TIE2-mutated patients, 11 (65%)

reached a ≥75%-improvement in symptoms with a median time to reach this response of 3 months (range 1-24 months). Among the 12 TIE2-mutated patients who completed the 2-year treatment, 5 had to restart sirolimus: 4 after 9 months and 1 after 18 months.

Conclusions

In conclusion, sirolimus is highly and rapidly efficient in PIK3CA-mutated patients; however, these patients who completed the 2-year treatment had to more rapidly restart sirolimus compared to TIE2-mutated patients.

Central conducting channel anomaly with associated PI3KCA variant presenting with rectovaginal leak

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Objectives: We describe a case of central conducting channel anomaly (CCLA) treated by embolization and targeted therapy.

Methods: A 9 year-old female presented with a 3 year history of protein-losing enteropathy with drainage of 1-2 L/day of chylous fluid from rectum and vagina, and severe abdominal pain.
Initial imaging showed extensive abdomino-pelvic lymphatic malformation. As features were consistent with CCLA, the patient was treated with sirolimus (target level of 10-13 ng/mL), but despite a response in leakage, this was discontinued due to adverse effects.
MR lymphangiogram by mesenteric injection showed channels leaking into the sigmoid colon and the vaginal vault. She then underwent laparotomy for glue embolization of pelvic lymphatic conglomerates and a lympho-venous anastomosis of a large retroperitoneal lymphatic channel to the right gonadal vein. Sigmoidoscopy with lymphatic dye injection was performed to guide the intervention and perform a rectal biopsy.

Results: High-coverage exome sequencing of DNA from biopsy identified a PI3KCA pathogenic variant c.3129G>A:p.M1043I at a 5.6% frequency. Shortly after the procedure, the leak resolved completely. But 2 weeks later, she developed severe abdominal pain, nausea, and anorexia, which required multiple admissions to hospital.
Alpelesib at 100 mg daily was initiated and well-tolerated. Her abdominal pain was controlled after consultation of clinical psychology and integrative health.

Conclusions: We describe multi-disciplinary management of a patient with CCLA, with surgical intervention, embolization, and targeted therapy. Molecular profiling of lesional tissue can provide crucial data to guide targeted therapy.

Lower limb overgrowth associated with first toe undergrowth in PIK3CA patients

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Objectives
PIK3CA-related overgrowth syndrome (PROS) include a heterogeneous group of disorders characterized by segmental overgrowth secondary to somatic mosaic activating mutations in PIK3CA. Segmental undergrowth is more uncommon and has been less studied but mutations in PIK3CA have also been found. With this in mind, we have noticed a group of PROS patients that present an undergrowth component associated with their focal overgrowth.

Methods
Retrospective review of PROS patients presenting overgrowth of the lower limb and undergrowth of the ipsilateral first toe was performed.

Results
Seven patients were included, four women and three men with a median age of 17 years. All patients presented a PROS phenotype with overgrowth of the lower limb and undergrowth of ipsilateral first toe. PIK3CA mutation was confirmed in 5 patients. Patients underwent multiple treatments, currently one patient is taking sirolimus, four are receiving alpelisib, one is asymptomatic and one died due to pulmonary thromboembolism.

Conclusions
Mutations in the same gene can create different phenotypes depending on the time and place in the gene. As far as we know, there have not been described opposing phenotpyes in the same patient. The presence of undergrowth in our series of PROS patients may be explained by genetic, embryogenic, maternal or placental factors but needs to be further investigated.

Sirolimus for in utero management of large fetal LM

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Objectives:
Lymphatic malformation (LM) is known to be caused by PIK3CA somatic mutations. We report the case of a fetus with a large LM involving the cervicofacial area with potential airway obstruction and extension into the mediastinum diagnosed at 16weeks of gestation.

Methods:
The parents had obtained agreement for medical pregnancy interruption. They consulted us for discussion of potential in utero therapies.
We explained the high risk of premature delivery or potential lethality of in-utero sclerotherapy or surgery. We alternatively discussed the possibility of trying maternal sirolimus treatment, as we had an actively recruiting clinical trial on sirolimus in vascular malformations, and since sirolimus crosses the placental barrier.
After agreement from our institution's ethical committee, and informed consent from both parents, the mother started sirolimus treatment at 22+5 weeks gestation. Important reduction of the LM was observed on Doppler US and on MRI after 3 and 7 weeks of treatment, respectively. Sirolimus was discontinued 5 days before delivery. At birth, the baby exhibited a cervicofacial LM but no respiratory distress.

Results:
She benefited postnatally from continued sirolimus treatment, sclerotherapy and partial surgical resection. NGS analysis identified a PIK3CA mutation.
She is now 4 years old with normal motor and intellectual development.

Conclusion:
LM is a rare malformation that can be life-threatening if located around the airway. Sirolimus, an mTor inhibitor, has emerged as a revolutionary add-on treatment for slow-flow malformations. This is the first LM treated in utero with sirolimus and with a sufficiently long-term follow-up to confirm its safety.

Novel, highly potent PI3Kalfa covalent inhibitors deconvolute class I PI3K isoform signaling

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Objectives: here, we present the generation of novel, drug-like, highly selective covalent PI3Kalfa inhibitors (19 and SAR) suited to decipher PI3Kalfa signaling [1].

Methods: HPLC-based chemical reactivity and TR-FRET assays were used to determine kchem, kinact and Ki of covalent inhibitors targeting PI3Kalfa. Cellular NanoBRET drug-target engagement revealed inhibitor diffusion rates and on target selectivity. Efficacy in cell lines with and without PI3Kalfa mutations was evaluated in drug washout, PI3K pathway monitoring and time resolved single-cell based FOXO translocation and proliferation assays to document long term inhibition of PI3Kalfa and its effects on cell fate.

Results: NanoBRET data with Cys862Ser mutant PI3Kalfa confirmed highly selective on-target reactivity of 19. Cells with mutated PI3Kalfa (MCF7, T47D, SKOV3) showed a persistent suppression of protein kinase B (PKB/Akt) phosphorylation after washout of compound 19, while PI3K signaling recovered rapidly with BYL719 and non-covalent structural analogs of 19. Recovery also occurred in PTEN null PC3 and A2058 cells treated with 19 due non-PI3Kalfa-mediated increase in PtdIns(3,4,5)P3. In PI3Kalfa mutant cells, FOXO1 remained nuclear even after removal of 19, confirming a persistent PI3Kalfa inhibition. Importantly, 19 provided a considerable gain in potency over BYL719, matched BYL719 in cellular diffusion rates and outperformed the prototype covalent PI3Kalfa inhibitor CNX-1351[2] in on-target reactivity, potency, stability and solubility.

Conclusions: Our results constitute a major step towards a drug-like covalent PI3Kalfa inhibitor for an unprecedented local and temporal control of PI3Kalfa-dependent oncogenicity and vascular malformation.

[1] Wymann Nat. Rev. Mol. Cell Biol. 2008.
[2] Nacht J. Med. Chem. 2013.

PIK3CA p.H1047L causes lymphatic dysplasia that can be improved with mTOR inhibition

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Objective: Lymphatic malformations (LM) are debilitating congenital lesions that arise from abnormal development of the lymphatic system. We saw a 12-year-old male patient presenting with microcystic and macrocystic LM of the cheek. At 9 months of age, a large laryngeal cyst obstructing his airway was removed. Although he has had multiple sclerotherapies, he has a persistent LM of his cheek. Genetic analyses and functional studies were undertaken to understand the etiology of his disease and identify targeted therapies.

Methods: Immunohistochemistry and a somatic gene panel for overgrowth and vascular malformations were performed using LM tissue. Sprouting of spheroids comprising transduced human lymphatic endothelial cells was used to model three-dimensional lymphangiogenesis in vitro. Gene expression in zebrafish using an mrc1a promoter was used to model lymphangiogenesis in vivo.

Results: Histology revealed irregular D2-40 and CD31 positive lymphatic channels. A somatic activating PIK3CA variant (c.3140A>T:p.H1047L) was identified in patient tissue. Expression of the variant in lymphatic endothelial cells was associated with more capillary-like structures emanating from spheroids in vitro, and with dilation of the thoracic duct and posterior cardinal veins in zebrafish. mTOR inhibition was effective in rescuing the hyperproliferative sprouting phenotype observed in vitro.

Conclusions: Persisting microcystic LM in a 12-year-old patient bearing the PIK3CA p.H1047 variant is likely caused by hyperactivation of the PI3K/AKT/mTOR signaling pathway. Preclinical modeling of lymphangiogenesis recapitulated his disease phenotype and identified mTOR as a potential therapeutic target for the treatment of his condition and others with LM.

Expert consensus on the testing and medical management of PIK3CA-related overgrowth spectrum

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Objectives: PIK3CA-related overgrowth spectrum (PROS) disorders are caused by somatic PIK3CA gene mutations. PI3K inhibitors are under investigation; early evidence shows they may be effective for patients with PROS. We conducted an expert RAND/UCLA Delphi panel to update 2015 guidelines and describe PROS severity classification, testing, and medical management.

Methods: We convened a diverse, experienced 13-member panel and reviewed evidence on PROS diagnosis and treatment. We collaboratively developed a rating form made up of 217 clinical scenarios having mild/moderate/severe presentations based on functional impairment, a reduction in quality of life, and risk of death. Before and after a virtual meeting, panelists were asked to rate each scenario's disease severity and the appropriateness of whether or not to test for a mutation and of prescribing mTOR/PI3K/AKT inhibitors.

Results: The panel developed clinical presentations and endorsed the disease severity framework. Panelists agreed it is appropriate to test for a PIK3CA mutation in every moderately/severely affected patient. Panelists agreed that it may be appropriate to consider an mTOR inhibitor in some severely affected patients and some moderately affected children or adolescents/adults with progressive disease. Panelists also agreed it may be appropriate to consider PI3K/AKT inhibitors in severely affected patients with a confirmed mutation or without a confirmed mutation but with progressive disease. The panel did not come to a consensus on the use of PI3K/AKT inhibitors in mildly/moderately affected patients.

Conclusions: These recommendations represent the consensus of 13 experts informed by literature and experience. Future research should validate this guidance using clinical data.

Mechanisms of Disease Across PIK3CA-Related Conditions: A Plain-Language Summary

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Objectives: PIK3CA-related overgrowth spectrum (PROS) conditions are rare and complex, and medical literature on this topic is limited and not primarily intended for the general public. Therefore, there are unmet educational needs for people with PROS conditions and their families. Here we review the biological cause of PROS conditions in accessible and plain language.

Methods: This plain-language summary is based on available medical literature.

Results: The diversity of features across PROS conditions makes it challenging to correctly diagnose. Therefore, many people are diagnosed by hallmark features and symptoms (CLOVES syndrome or Klippel-Trenaunay syndrome, for example). Despite this diversity of features, most PROS conditions are caused by changes, or mutations, in a gene called PIK3CA. This gene provides instructions to make a protein called PI3K-alpha. In people with PROS, PIK3CA mutations cause PI3K-alpha to be activated. This causes abnormal growth of tissues such as blood vessels, lymphatic vessels, muscles, and bone. Although the same types of mutations seen in PROS are found in cancer, they are different conditions/diseases. In PROS, mutations in PIK3CA are not inherited, they occur sometime after fertilization. Mutations that occur very early in development of the fetus typically lead to widespread and severe conditions. Mutations that occur later may lead to more local abnormal growth and milder conditions. Overall, the type, timing, and location of PIK3CA mutations determine the clinical features for each person.

Conclusions: Although PROS conditions are complex and affect different people in different ways, they share a common genetic cause.

EPIK-P1: Retrospective Chart Review of Patients With *PIK3CA*-Related Overgrowth Spectrum Who Received Alpelisib as Part of a Compassionate Use Program

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Background: Alpelisib, a PI3Kα inhibitor, showed promising results in patients (n=19) with PROS (Venot, *Nature* 2018).

Methods: EPIK-P1 is a non-interventional, retrospective medical chart review of patients (≥2 years) with PROS treated with alpelisib through a managed access program. Patients had severe/life-threatening conditions, confirmed *PIK3CA* mutation, and received ≥1 dose of alpelisib ≥24 weeks before data cutoff. The primary endpoint assessed patient response (yes/no) to treatment (≥20% reduction in sum of target lesion[s] volume at week 24); complete cases had scans at index and week 24. Secondary endpoints assessed safety and clinical benefit.

Results: Data were abstracted from 57 patients. Median exposure was 18.1 months. In the primary endpoint analysis, 12/32 patients with complete cases (37.5%) were responders; 23/31 patients with imaging (74.2%) showed reduction in target lesion volume, mean±SD reduction 13.7±18.9%. At week 24, proportion of patients (in the full-study population) with improvement in the most frequent PROS-related symptoms/signs was pain 90.9% (20/22), fatigue 76.2% (32/42), vascular malformation 78.9% (30/38), limb asymmetry 69.0% (20/29), and disseminated intravascular coagulation 55.2% (16/29). Adverse events (AEs) and treatment-related AEs were experienced by 82.5% (47/57) and 38.6% (22/57) of patients, respectively; most common treatment-related AEs were hyperglycemia (12.3%) and aphthous ulcer (10.5%). No deaths were reported.

Conclusions: Real-world data demonstrate alpelisib is clinically effective and well tolerated in patients with PROS. Previously presented at European Society of Medical Oncology 2021, FPN:LBA23, Guillaume Canaud et al. Reused with permission.

Thinking Zebras when Hearing Hoofbeats: Engaging Rare Disease in Medical School Curricula

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Objectives: Medical school curriculum emphasizes common diseases. Yet, there are over 300 million individuals living with rare diseases worldwide, representing a need for change in medical school curricula. The Penn Rare Disease Interest Group (Penn RareDIG) aims to increase awareness of rare diseases among medical students and enrich coverage of rare diseases. Penn RareDIG also aims to foster a community in which medical students can advocate on behalf of individuals with rare diseases.

Methods: A medical school elective on rare disease advocacy will begin in the fall of 2021. The elective will feature lectures with rare disease patients, families, clinicians, and researchers. Additionally, a rare disease curriculum map will assess gaps in the core medical school curriculum. Finally, pictorial infographics and short patient video blogs on rare diseases will be regularly shared via email and social media to disseminate information in a quick and accessible manner.

Results: A pilot seminar series in the spring of 2021 included speaker events highlighting Kaposiform Lymphangiomatosis, TBCK-related encephalopathy, and short bowel syndrome. These events ran alongside relevant curriculum blocks, hematology/oncology, neurology, and gastroenterology, respectively. In parallel with the endocrinology and gastroenterology blocks, supplementary infographics on McCune-Albright syndrome and achalasia were disseminated.

Conclusions: Future clinicians will encounter patients with rare diseases, regardless of specialty. Addressing curricular gaps can empower medical students to better care for rare disease patients, and in turn, improve the healthcare experiences of individuals with these illnesses. Further curricular engagement plans include collaboration with nursing students, advocacy, and research opportunities.

Childhood Surgical Toll of *PIK3CA*-Related Overgrowth Syndrome Mandates Early Initiation of Targeted Medical Therapy

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Background. *PIK3CA*-related overgrowth syndrome (PROS) clinical presentation is variable and ranges from macrodactyly to life-threatening, debilitating CLOVES or MCM syndromes. Starting in infancy, patients undergo multiple surgical procedures to improve their quality of life and normal development.

Objective. To retrospectively analyze the surgical history targeting PROS-related malformations of all patients with a proven somatic mosaic mutation in *PIK3CA* followed in our institution's Vascular Anomalies Center.

Results. We studied 26 patients, ranging from age 6 months to 20 years old. The population included 11 CLOVES, 8 MCM (3 pure, 3 MCM/CLOVES, and 2 MCM/facial lipomatosis), 3 Klippel-Trenaunay syndrome, 2 lymphatic malformations, and 2 others (macrodactyly and macrodactyly/aortopulmonary collaterals). There were 69 operations, not including biopsies for genetic testing or sclerotherapy/endovascular procedures, performed on 20 patients (6 patients had none). Sixty-three procedures were performed before 15 years of age. Surgical types: 34 debulking interventions, 10 amputations, 10 orthopedics and reconstructions, 3 hemispherectomies for intractable seizures, and 12 others. The most significant number of procedures per patient was CLOVES phenotype (up to 20 per patient), and the lowest number was for pure MCM (none).

Conclusion. Congenital distortion of anatomic landmarks and rapid progression during early childhood forces patients with PROS to undergo multiple surgical interventions in early life with inherent high complication risks. Evaluation for somatic genetic alterations of affected tissue and initiation of targeted medical therapy during the first years of life aimed at preventing invasive procedures should become a main priority in the field.

Multiple congenital and postnatal disease states modeled by somatic mosaicism for constitutively active Pik3ca signalling in neural crest-derived lineages

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Ectopic expression of driver oncogenes can confer competitive growth advantages to individual cells, sometimes at the expense of the organism. A recurrent missense mutation (H1047R) in the phosphatidylinositol 3-kinase (PI3K) catalytic subunit p110-α, encoded by the PIK3CA gene, leads to its constitutive activation in multiple cancers. The same mutation has also been identified in a spectrum of congenital malformations and tumours, commonly inducing overgrowth in components of skin, brain, adipose, connective, musculoskeletal tissues and/or blood/lymphatic vessels, among others.

OBJECTIVES: To test the hypothesis that PI3K activation at different times of embryogenesis in neural crest (NC) lineages models specific aspects of PROS (PIK3CA-related overgrowth spectrum) disorders. METHODS: Phenotypic characterization by histology, immunofluorescence and micro-CT of genetically modified mice after restricted expression of activated Pik3ca H1047R to specific derivatives of neural crest cells over time.

RESULTS: Early expression of Pik3ca H1047R in the entire neural crest lineage leads to severe craniofacial defects, including fully penetrant cleft palate, megalencephaly, a hypoplastic pituitary gland and venous malformations in the head, neck and heart, concluding in perinatal death. Later expression in more restricted neural crest-derived lineages induce phenotypes compatible with survival but are associated with tumorigenesis at various life stages.

CONCLUSIONS: Neural crest-derived cells expressing Pik3ca H1047R are subject to cell-autonomous overgrowth and seem to also exert inductive effects on developing tissues, particularly craniofacial. Given the phenotypic overlap with conditional Braf(V600E) induction, these models will allow further characterization of the timing and molecular consequences in utero of interaction between PI3K and contingent signaling pathways.

Segmental lymphatic malformation of the upper limb and chest wall mimicking congenital melanocytic nevus, with ipsilateral pleural effusion

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Introduction: Primary lymphatic malformations that affect both skin and viscera are diffuse in nature such as generalized lymphatic anomaly and Kaposiform lymphangiomatosis except for Gorham-Stout disease which can be focal with marked osteolysis. We present a child with hyperpigmentation and hypertrichosis as the manifestation of lymphatic malformation in the skin extending to underlying tissues and adjacent pleura, in a segmental nature, unlike any of the above reported entities.

Case: A 2.5-year-old boy with recurrent right sided pleural effusion for 1 month was noted to have progressive hyperpigmentation and coarse terminal hair with skipped areas in a segmental pattern on the right upper limb and the axilla since 7 months of age. Differentials included congenital melanocytic nevus, smooth muscle hamartoma and plexiform neurofibroma. Chest radiograph and ultrasound examinations revealed gross right sided pleural effusion with collapsed underlying lung. MRI revealed confluent cystic spaces in the subcutaneous, intra-muscular and inter-muscular planes of the right upper limb and chest wall along with intra-osseous involvement of the right upper limb without osteolysis. On aspiration, the effusion was transudative and non-chylous. Skin biopsies with immunohistochemistry for D2-40 confirmed the lymphatic malformation. Thus, a diagnosis of lymphatic malformation of the upper limb and chest wall with ipsilateral pleural involvement was made and the child was initiated on sirolimus.

Conclusion: Hyperpigmentation and hypertrichosis without overt microcystic disease is an unusual presentation of lymphatic malformation in the skin. Cutaneous lymphatic malformations even when segmental require evaluation of concomitant malformations in the adjacent tissues and organs.

Atypical PIK3CA positive hemangioma refractory to propranolol

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OBJECTIVE: Retrospective report of an atypical PIK3CA positive anomaly refractory to propranolol.

METHODS: IRB approved chart review.

RESULTS: A term female born with a combined superficial and deep, dark red, raised vascular lesion lateral to right eye. The lesion demonstrated marked growth within first weeks of life. She was started on oral propranolol 3mg/kg/day with a working diagnosis of infantile hemangioma. While ultrasound and clinical findings remained consistent with infantile hemangioma, proliferation continued despite therapeutic dosing. At five months, mass effect of the lesion in the right upper eyelid threatened a lateral visual field cut and she had a near total surgical resection. Histology demonstrated areas consistent with infantile hemangioma with additional atypical areas of larger serpiginous, ectatic vessels. Staining showed strong immunoreactivity for CD34, CD31, GLUT-1, and immunonegative for D2-40. Genetic sequencing on resected tissue was positive for a likely pathogenic somatic PIK3CA variant (c.3141T>G, p.His1047Gln) at 8.3% allele frequency. This variant located in a hotspot recognized to activate downstream PI3K signaling has been reported in patients with PIK3CA related overgrowth syndromes. Despite continued postoperative propranolol, the lesion has new mass effect over the right cheek, and she has started a trial of oral sirolimus.

CONCLUSIONS: This rapidly growing anomaly was refractory to propranolol, demonstrated atypical mixed histologic features with a staining pattern like infantile hemangioma, but has genetics supporting a PIK3CA mosaicism. The case hybridizes findings of vascular tumor and a vascular malformation and demonstrates the impactful role of pathologic and genetic evaluation in guiding diagnosis and therapy.

Developmental venous anomaly in PIK3CA¬related overgrowth spectrum disorder.

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Objectives: We report two patients with developmental venous anomaly (DVA) in the setting of PIK3CA-related overgrowth spectrum disorder (PROS).

Methods: Patients were evaluated for orbital/periorbital lymphatic anomalies. Magnetic resonance imaging (MRI) was obtained on both patients. Somatic genetic testing was performed on lymphatic tissue from both patients for 44 genes related to lymphatic and vascular malformations.

Results:
Patient 1 has a periorbital microcystic lymphatic anomaly. Lymphatic malformation was treated with sirolimus and sclerotherapy. MRI identified symmetric bilateral prominent cerebellar DVA with drainage into a distended vein of Galen and dural venous system. Pathology noted findings compatible with a lymphatic malformation. Genetic testing revealed a somatic pathogenic PIK3CA variant (c.1633G>A, p.Glu545Lys) present in tissue (variant allele fraction (VAF): ~3%).

Patient 2 had a left orbital vascular malformation that ultimately resulted in left globe enucleation. MR angiogram noted DVA described as multiple anomalous veins draining from region of left temporal lobe posteriorly into the sagittal sinus. Pathology noted a vascular malformation with findings suggestive of lymphatic malformation. These findings were suggestive of a cerebrofacial vascular metameric syndrome. Somatic pathogenic PIK3CA variant (c.1624G>A, p.Glu542Lys) was present in tissue (VAF: 2%).

Both variants have previously been reported in PROS.

Conclusions:
Further research is needed to understand the molecular mechanisms of DVAs. We propose PIK3CA as a novel cause of DVA, particularly in the setting of ipsilateral lymphatic or vascular malformations, although more cases are needed to support this proposal.

Is this the answer? A variant of uncertain significance in PIK3CA in a patient with generalized lymphatic anomaly

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Objectives: Somatic pathogenic variants in PIK3CA are a well-known cause of generalized lymphatic anomaly (GLA), however variants of uncertain significance (VUS) complicate diagnosis and treatment. We present an individual with GLA and a rare somatic VUS in PIK3CA to solicit similar cases.

Methods: Clinical care was performed. Consent was provided. Functional studies in lymphatic endothelial cells and zebrafish are ongoing to evaluate the pathogenicity of the variant.

Results: After birth, the patient was found to have lymphatic malformations (LMs) involving the stomach, intestines and neck. At 2 months old, she was started on sirolimus. At 16 months old, she had exacerbation of disease characterized by lymphedema and protein losing enteropathy (PLE). Dynamic contrast magnetic resonance lymphangiography was consistent with hepato-mesenteric lymphatic obstruction likely secondary to a large mesenteric LM with microcystic bowel wall involvement of the duodenum and transverse colon. A low-fat diet was initiated. Her PLE resolved. Her albumin normalized. A gene panel identified a somatic VUS in PIK3CA (c.1571G>A:p.Arg524Lys, allele frequency 2.8-3.7%) from genomic DNA isolated from blood and pleural fluid. The variant has been reported in oral and esophageal neoplasia but not in lymphatic disease. The VUS is absent from large populations. However, in silico analyses suggest it is benign. Her recent MRI showed the LM increased increase in size. She remains without PLE symptoms. Due to the uncertainty of the variant, we have not transitioned her to alpelisib.

Conclusions: In conclusion, a rare somatic VUS in PIK3CA has complicated medical therapy for this patient with GLA.

Benign tumors in children associated to PIK3CA mutation

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Objectives
Somatic mosaic activating mutations in PIK3CA are found in cancer, lymphatic and venous malformations, and PIK3CA-related overgrowth spectrum (PROS) disorders. These variants have also been found in some benign pathologies such as seborrheic keratosis, endometriosis or PROS-related lipomas, but to our knowledge they have not been described in other benign tumors.

Methods
Retrospective review of patients presenting benign tumors associated to PIK3CA mutations was performed.

Results
Six patients were included in the study with benign tumors associated to four different PIK3CA variants. Two patients presented a congenital hepatic hemangioma, one had multiple cutaneous congenital hemangiomas, one had a presacral ganglioneuroma in the context of CLAPO syndrome, one presented a left renal vascular hamartoma also in the context of CLAPO syndrome and the last one underwent resection of a provisionally unclassified vascular tumor.

Conclusions
Mutations in PIK3CA have been thoroughly studied in cancer, vascular anomalies and overgrowth syndromes. We have found the same variants in some benign tumors, not previously associated to PIK3CA mutations. This connection needs to be assessed in larger series of patients so that, if confirmed, could allow these patients to benefit from targeted therapies as well. In fact, two patients in our group are currently been treated with alpelisib and sirolimus.

Structural Mechanism for PI3Kα H1047R Mutant Drug Discovery

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Phosphoinositide 3-kinase (PI3K) is the lipid kinase in PI3K/Akt/mTOR pathway, regulating cell proliferation and growth. The PI3Kα is the second highly mutated signaling proteins in cancer. H1047R in PI3Kα shows the highest frequency in cancer and PIK3CA-related overgrowth syndrome. To date, PI3Kα mutants are drugless. The available drugs target the wild-type PI3Kα. Their safety profiles are usually not sufficiently good, resulting in the strong adverse side-effects, limiting their applications. Over the past decade, drug discovery for PI3Kα mutants has been intensely sought-after. However, different from other protein kinases, the hotspot mutations in PI3Kα do not occur within or nearby the ATP pocket, making the design of PI3Kα mutant drugs highly challenging. To date, the structural principle for designing the drugs against PI3K mutants has been unavailable. Our work has uncovered the structural mechanism of PI3Kα activation upon nSH2 release at the atomic resolution, highlighting the significant conformational changes that promote the membrane interactions and PIP2 catalysis. The mechanism has been validated by a set of crystal structures and additional experimental data. We identified the structural features (a-loop and kα-11) that distinguish the inactive and active class IA PI3Ks. The movements of a-loop and kα-11 in the PI3Kα activation explain the aberrant activation of PI3Kα H1047R mutant in cancer and overgrowth syndrome. Our structural studies reveal a structural principle for PI3Kα H1047R mutant drug discovery, where maintaining the inactive conformation is the key.

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