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Genetics Corner: Klippel-Trenaunay Syndrome in an Infant with a Mosaic PIK3CA Variant, a Target for the Medical Treatment of Asymmetric Overgrowth

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Case Summary:

A 16-week-old late preterm female with Klippel-Trenaunay syndrome (KTS) was referred to the Genetics clinic. She was born at 36 wk 6 d gestation by spontaneous vaginal delivery to a 26-year old G2P1 mother, with good prenatal care and uncomplicated pregnancy. A 20 wk prenatal ultrasound exam was normal. All growth parameters were appropriate for gestational age: birth weight 3280 g, birth length 49.1 cm, and head circumference 32.7 cm. Apgar scores were 7 and 8 at 1 and 5 minutes, respectively.

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When she was examined in the newborn nursery, subtle hemihypertrophy of the right leg, a wide right foot with splayed toes, and a port-wine stain on the midback were appreciated. The genetics service was informally consulted, and a clinical diagnosis of Klippel-Trenaunay syndrome was made. A peripheral blood sample was collected from the affected right foot for *PIK3CA* gene analysis before discharge from the nursery. The baby has been well at home. Family history was noncontributory

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Figure 1. In the newborn period (Figure 1a), the right leg and foot were darker in color and subtly larger in circumference than the left leg. The right foot (Figure 1b) was larger and wider, and the right toes were larger and more splayed than the toes of the left foot. A port-wine stain (Figure 1c) extended to the right midaxillary line in the midback. It crossed the midline to the left midback in a few places.

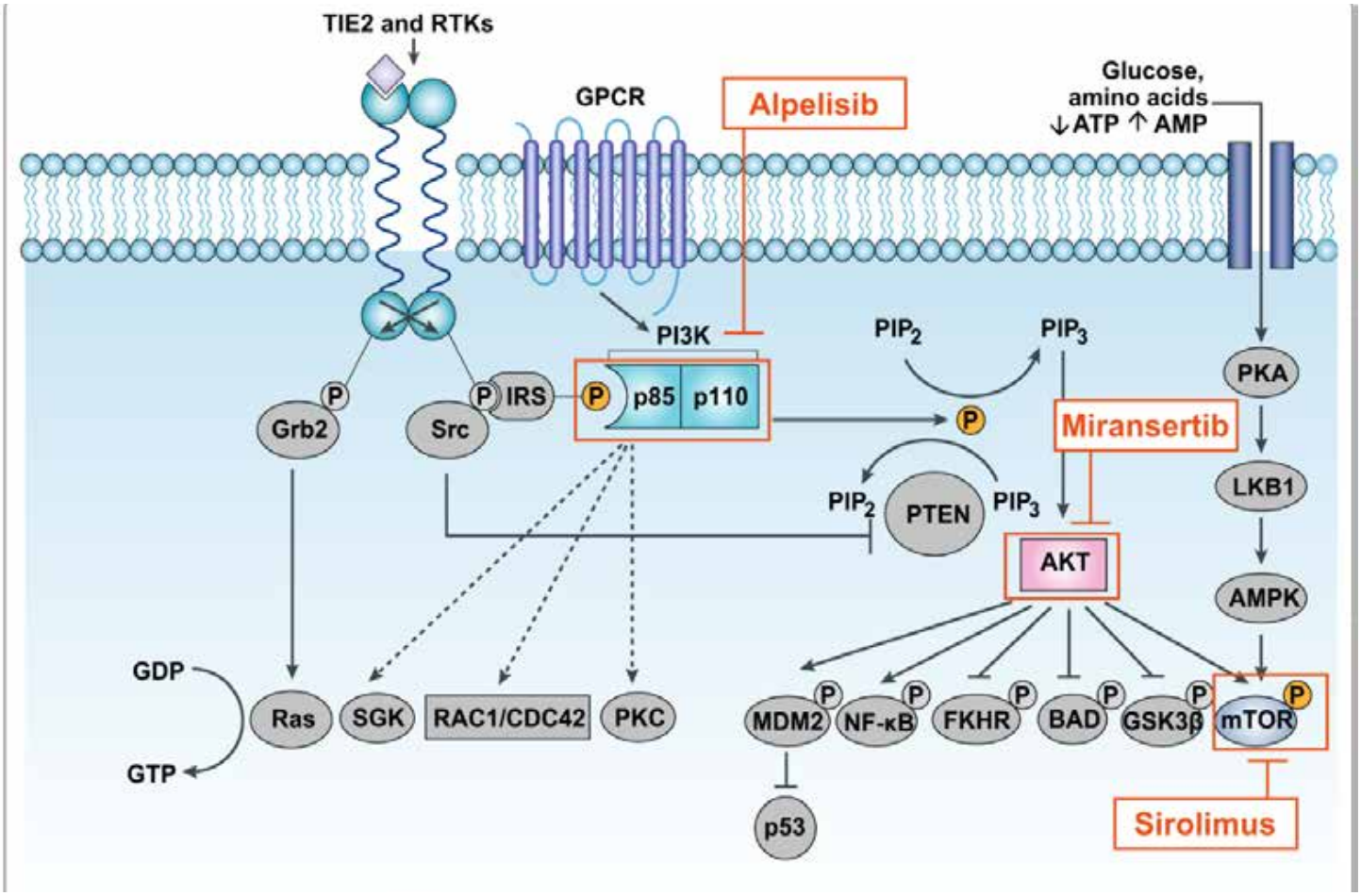

Figure 2. Drugs that inhibit the PIK3/AKT/mTOR pathway are shown in this illustration from Canaud (5). At the top of the pathway, alpelisib inhibits PI3K and diminishes activation of the downstream growth-promoting genes in the pathway. This is the basis for its therapeutic effect in PIK3CA-related overgrowth spectrum (PROS) disorders such as Klippel-Trenaunay syndrome.

In the last two months, the parents noted a new fatty prominence in the right lower quadrant that was soft and non-tender on examination at 16 weeks. There was also mild asymmetry and fatty enlargement of the right labia majora and right buttock. Otherwise, the baby was nondysmorphic. She was developing normally.

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Genetic analysis of peripheral blood (Perelman, U Penn) identified a heterozygous pathogenic mosaic (0.06%) variant in exon 2: c.317G>T (p.Gly106Val) in *PIK3CA*, consistent with a *PIK3CA*-Related Overgrowth Syndrome (PROS).

Discussion:

Neonatologists and pediatricians are familiar with Klippel-Trenaunay syndrome (KTS), a sporadic vascular malformation with asymmetric overgrowth, usually affecting a lower extremity, in an otherwise healthy newborn. Until recently, the genetic cause of this disorder was not appreciated, and there was no available medical therapy. Now that the cause of KTS is understood to be an activating gain-of-function variant in *PIK3CA* that responds to targeted medical therapy, it is time to reconsider our approach to KTS in the newborn period.

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KTS is one of the *PIK3CA*-Related Overgrowth Spectrum of disorders known as PROS (1). The pattern of overgrowth in PROS is tissue specific. Depending on the developmental fate of the embryonic cell that harbored the original *PIK3CA* pathogenic variant, the various PROS spectrum disorders present with strikingly different phenotypes ranging from bulky lipomatous lymphangiomas (CLOVES) to epidermal nevi to macrodactyly or hemimegalencephaly. KTS is one of the milder presentations of PROS.

The *PIK3CA* variant in PROS is a post-zygotic, somatic, and mosaic change that occurs early in a dividing cell in the developing embryo. The activating variant populates the cells that arise from the first affected cell, and the genetic change is not present in all cells of the body. The blood sample is often normal, and the mosaic *PIK3CA* variant is only detectable in fresh biopsy tissue from an affected body part. We were able to avoid an invasive procedure for this patient. We took a chance on this infant and ordered the blood to be drawn from the *affected* foot, hoping that some affected tissue from the skin or the venous wall might be collected with the blood sample. "Contamination" with a small amount of affected tissue might have been why we could detect low-level mosaicism in this blood sample. We did not, but perhaps in the future might draw a simultaneous blood sample from an unaffected limb to confirm that drawing the sample from the affected limb enhances the detection rate of low-level mosaicism. For now, it is just a hypothesis but one that bears testing.

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PIK3CA, a member of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway (Figure 2), acts as an oncogene in breast and other cancers. Because activation of this pathway promotes cell growth, proliferation, cell survival, and angiogenesis, PIK3CA has become a pharmacological target in treating *PIK3CA*$^{+}$ HR^{+} (hormone receptor)/HER2^{-} metastatic breast cancer. Alpelisib, an orally available α-selective PIK3CA inhibitor that blocks the PI3K/AKT/mTOR pathway, has effectively treated *PIK3CA*-positive breast cancer (2). The same specific activating variants in *PIK3CA* are often present in breast cancer and the bulky overgrown tissues in PROS. This led researchers to study alpelisib as a possible treatment for children and adults with PROS. These clinical trials have demonstrated that alpelisib effectively reduces bulky masses in severe overgrowth due to PROS (3,4). In April 2022, the FDA approved Alpelisib for use in adults and children over two years of age who have serious manifestations of PROS.

FDA approval was based on real-world evidence from the EPIK-P1 study, a retrospective chart review study showing patients treated with alpelisib experienced reduced target lesion volume and improved PROS-related symptoms and manifestations. Here is a summary of the EPIK-P1 study data submitted to the FDA but has not been published. The primary endpoint analysis conducted at week 24 showed that 27% of patients (10/37) achieved a confirmed response to treatment, defined as a 20% or greater reduction in the sum of PROS target lesion volume. Nearly three in four patients with imaging at baseline and at week 24 (74%, 23/31) showed some reduction in target lesion volume, with a mean reduction of 13.7%, and no patients experienced disease progression at the time of primary analysis. Additionally, at week 24, investigators observed patient improvements in pain (90%, 20/22), fatigue (76%, 32/42), vascular malformation (79%, 30/38), limb asymmetry (69%, 20/29), and disseminated intravascular coagulation (55%, 16/29). These improvements were observed in subsets of patients across the study population (n=57) who reported symptoms at baseline and at week 24. Professor Canaud discusses EPIK-P1 study data in a short video (6). A prospective trial, EPIK-P2, has already begun.

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We referred our patient to the Pediatric Hematology/Oncology service to discuss the benefits and limitations of alpelisib therapy, which would be available after age two years. We also recommended serial imaging studies for volumetric analysis prior to therapy to establish the size of the lesion at baseline and the rate of growth.

KTS is only one of the somatic overgrowth disorders that may be treated with drugs that target the PI3K/AKT/mTOR pathway. Vascular malformations in children often show mutations in *PIK3CA* or *AKT*. Drugs that target *PIK3CA*, *AKT,* and other downstream genes in the pathway may provide therapies to a group of somatic conditions that have not been previously amenable to treatment (7).

Practical applications:

1. Recognize that *PIK3CA*-related overgrowth spectrum (PROS) disorders include a wide variety of overgrowth phenotypes that differ based on the tissue-specific developmental fate of the original mutant cell. Klippel-Trenaunay syndrome (KTS) is probably the most common PROS disorder.
2. Appreciate that mosaic (somatic) heterozygous pathogenic activating *PIK3CA* variants stimulate growth pathways in affected tissues, which presents a target for treatment.
3. Expect more overgrowth syndromes to be treated with drugs, such as alpelisib, originally developed to treat cancer.
4. Understand that documentation of a *PIK3CA* variant is necessary in KTS and other PROS disorders in order to offer therapy that specifically blocks the downstream effects of the activating *PIK3CA* gene variant.
5. Order *PIK3CA* gene testing in all infants with features of KTS or asymmetric overgrowth that suggests a PROS disorder. A peripheral blood sample may not detect the *PIK3CA* variant responsible for asymmetric overgrowth in a child with PROS. We speculate that collecting a blood sample from an affected

limb may enhance the detection of a mosaic *PIK3CA* variant but submitting a fresh tissue sample from an affected region for gene testing is the most reliable approach.

6. Refer patients with KTS or other PROS disorders for genetic and oncology consultations

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