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A Phase II double-blind multi-center, placebo-controlled trial, to assess the efficacy and safety of alpelisib (BYL719) in pediatric and adult patients with Megalencephaly-CApillary malformation Polymicrogyria syndrome (MCAP) – the SESAM study protocol

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A Phase II double-blind multi-center, placebo-controlled trial, to assess the efficacy and safety of alpelisib (BYL719) in pediatric and adult patients with Megalencephaly-CApillary malformation Polymicrogyria syndrome (MCAP) – the SESAM study protocol

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Abstract:

Introduction

The MCAP syndrome (Megalencephaly Capillary malformation Polymicrogyria) results from mosaic gain-of-function *PIK3CA* variants. Main clinical features are macrocephaly, somatic overgrowth, neurodevelopmental delay, and brain anomalies. Alpelisib (Vijoice®) is a recently FDA-approved PI3Kα-specific inhibitor for patients with PIK3CA-related overgrowth spectrum (PROS). During its development, in patients with MCAP subgroup of PROS, there was no specific, standardised evaluation of the effect on neuro-cognitive functioning. Moreover, it remains unknown if the molecule crosses the blood-brain barrier. Our objective is to evaluate the efficacy of a 24-month treatment with alpelisib on adaptive behaviour in patients with MCAP syndrome.

Methods and Analysis

SESAM is an industry-sponsored two periods multi-centre French academic phase II trial, with a 6 months double-blind, placebo-controlled period followed by open label period. Primary endpoint is a ≥4 points improvement in the Vineland II Adaptive Behavior Scale (VABS), 24 months after treatment initiation. Secondary objectives are safety, VABS improvement at 6 months, impact on quality of life, epilepsy and hypotonia. Twenty patients aged 2 to 40 years old, with a MCAP diagnosis and neurodevelopmental disorders of various degrees, will be followed monthly in local centres, centrally assessed (clinical, biological, neuropsychological and functional evaluation) at baseline and every 6 months. Patients will be evaluated by volumetric MRI at baseline and at 24 months. An optional lumbar puncture will be performed to investigate blood-brain barrier crossing. Inclusions are expected to be completed by March 2024, with the end of follow-up in September 2026.

Given the efficacy of alpelisib in patients with PROS, if the drug crosses the blood-brain barrier, we can expect a clinical benefit for patients with neurocognitive disorders.

Ethics and dissemination

Ethical approval was given by CPP Sud-Ouest et Outre-Mer I (reference: 2022-500197-34-01). Findings from this study will be disseminated via publication, reports and conference presentations.

Trial registration Number (ClinicalTrials.gov)

NCT05577754

Keywords:

Neurodevelopmental disorders, alpelisib, therapeutic trial, PIK3CA, MCAP

Word count: 3822 words

Strengths and limitations of this study:

- The SESAM trial is the first evaluation of alpelisib dedicated in patients with MCAP using neurocognitive endpoints.
- The passage of alpelisib through the blood-brain barrier will be evaluated in the SESAM trial.
- The two-period design (double blind placebo-controlled period followed by open-label period) has been chosen to comply with the best quality methodological standards.
- Heterogeneity in patient's clinical presentation represents a challenge in the interpretation of the results.
- No international consensus exists on the scales to be used in clinical trials in patients with neurocognitive disorders.

INTRODUCTION

Segmental overgrowth disorders (SODs) are rare conditions usually characterized by abnormal and asymetric growth of some parts of the body. This excessive tissue growth is caused by an overactivation of the cell proliferation mechanism. Mosaic activating variants in the p110α catalytic subunit of phosphatidylinositol-3 kinase (PI3K; encoded by the *PIK3CA* gene) have been identified in a subset of SODs. The PI3K-AKT-mTOR is a critical signalling pathway in regulating proliferation, survival and cell growth. Activating variants in *PIK3CA* lead to increased PI3K-AKT-mTORC1 axis activation, which in turn promotes excessive growth in affected tissues (1-6).

The PIK3CA-related overgrowth spectrum (PROS) is a congenital condition with progressively asymmetric overgrowth which can begin in the antenatal period. This disease is wide-ranging, and depends upon the timing of the founder mutation in embryogenesis (6-9). Depending on the clinical presentation (segmental body overgrowth or brain disorder), three main disease subgroups can be distinguished: the CLOVES syndrome (Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevi, Scoliosis/Skeletal/Spinal anomalies), the Klippel-Trenaunay syndrome and the MCAP syndrome (Megalencephaly Capillary malformation Polymicrogyria). CLOVES clinical presentation ranges from isolated digit enlargement to extensive overgrowth of limbs, thorax/abdomen and/or face. It may be accompanied by vascular or lymphatic malformations, epidermal nevi and spinal anomalies. Associated morbidity can be highly variable, but can include functional impairment, debilitating haemorrhages and thromboses, and, in some cases, can be lethal. For patients with Klippel-Trenaunay syndrome, vascular malformations are often in the foreground, associated with soft tissue and bone hypertrophy, and involve one limb, most often the lower limb. MCAP is

characterized by megalencephaly (large head), capillary malformation of the skin (middle face, limbs and trunk), abnormalities of the extremities and possible abnormalities of the brain structure (Chiari malformation, hydrocephalus, polymicrogyria) (9, 10). Megalencephaly is present at birth, and the brain continues to develop gradually during the first postnatal years with a relative stabilization with age, although remaining larger than normal. MCAP patients may experience impaired cognition, hypotonia, variable intellectual deficiency and seizures. Abnormalities of the brain structure may be present (hydrophalus, Chiari malformation and polymicrogyria in particular). Asymmetry of some body parts may also exist, as well as skin manifestations. Cardiac and genitourinary abnormalities have been reported in rare cases. The current diagnosis is established through a clinical evaluation and genetic testing on the affected tissues. In rare cases, the *PIK3CA* variant is present in all cells and not at a mosaic state. Magnetic resonance imaging (MRI) is used to identify and monitor brain abnormalities. The French National Protocol for Diagnosis and Care for MCAP syndrome (11) and an international expert consensus statement for standardizing care for individuals with *PIK3CA*-related disorders have been published (12). As patients may be misdiagnosed, the true prevalence of the disease is not well known. But in France, in 2021, more than 60 MCAP patients had already been genotyped. Variability in the degree of neurocognitive manifestations is considerable, ranging from mild learning disabilities to profound intellectual disability, in some cases associated with epilepsy (10).

The natural history of PROS shows that most of the overgrowth progression occurs during early childhood, emphasizing the need to assess the potential benefit of early treatment in paediatric patients with PROS. It could avert associated complications and/or surgery by decreasing disease progression at this active stage.

Interestingly, drug treatments that specifically inhibit the p110α catalytic subunit of PI3K have been developed in oncology in case of tumors with *PIK3CA* gain-of-function variants. Alpelisib (Vijoice®, Novartis Pharmaceutical) have been authorized in metastatic breast cancer (13, 14), and has therefore been investigated through drug repurposing in PROS, in a case series of 19 French patients under a compassionate approach, including 2 with MCAP (15). After encouraging results in safety and efficacy, Novartis Pharmaceutical started the EPIK program. The EPIK-P1 (NCT04285723) is a real-world study for demonstrating clinical benefit in people with PROS, and EPIK-P3 (NCT04980833) a phase II study to assess long-term safety and efficacy of alpelisib in people with PROS who participated in EPIK-P1. The ongoing international prospective phase II double-blind, randomized, placebo-controlled study clinical trial (EPIK-P2, NCT04589650), is assessing efficacy, safety and pharmacokinetics of alpelisib in pediatric and adult patients with PROS. Positive preliminary results from EPIK-P1 led the FDA to grant early April 2022, an accelerated approval for alpelisib (Vijoice®) in PROS, including MCAP, for patients of 2 years of age and over, based on the efficacy (defined as a ≥20% reduction from baseline in the sum of measurable target lesion volume) observed in 37 patients from EPIK-P1 after 6 months of treatment (16). However, these therapeutic studies preferentially targeted patients with CLOVES or Klippel-Trenaunay syndromes, and endpoints were not designed to assess neurocognitive improvement. Current data on alpelisib efficacy and safety in MCAP patients solely comes from compassionate use, which fails to demonstrate a clear benefit of the treatment on neurocognitive symptoms (15, 17). There is even no proof that alpelisib crosses the blood-brain barrier.

To assess the clinical benefit of Alpelisb in MCAP patients, it was necessary to construct a clinical trial evaluating specifically neurocognitive functions. Based on the clinical and radiological evaluation of 33 French patients with MCAP syndrome (10), we designed the

SESAM study to demonstrate the safety and efficacy of alpelisib in these patients, and to assess its passage across the blood-brain barrier.

METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

Study Setting

This study is promoted, partly funded, and coordinated by Dijon Bourgogne University Hospital. Drug supply and part of funding was supported by Novartis Pharmaceutical. It includes pediatric and adult patients covered by national health insurance. To minimize the logistic burden for the patients, inclusion and safety visits are performed in their local hospitals (9 sites in total). Two evaluating sites (Dijon Bourgogne University Hospital and Paris Necker Hospital) are in charge of the baseline and assessment visits.

Study design, participant, randomisation

Study design. The SESAM trial is a two-period multi-center Phase II trial, with 6 months double-blind, placebo-controlled period followed by an open label period (Figure 1). Patients and their legal representative are given by the investigator a consent form adapted to their age and ID severity. A separate and optional consent for lumbar puncture is also proposed. After validation of the screening exams at the inclusion visit, patients are randomized to take alpelisib (250mg/day for adults or 50mg/day for children) or placebo during 6 months, with a 1:1 ratio. A first evaluation will be performed at 6 months (secondary objective 1) to determine the response status and patients will be unblinded once the evaluation is done. Patients completing the double-blind phase will then be entered in an open-label phase as follows: patients on placebo will switch to alpelisib (250mg/day for adults or 50mg/day for

children), responders to alpelisib will continue alpelisib at same dose during 18 months, and non-responders will have their dose increased (300mg/day for adults, 125mg/day for children ≥6 years old only). Dose increase is not permitted for children aged 5 years and below. Non-responders are patients who did not experience sufficient clinical benefit (based on overall clinical response assessed by the investigator) and with no safety/tolerability concerns which may preclude from treatment continuation at higher dose level. Patients will undergo additional evaluation visits every 6 months, and a final evaluation visit after 24 months of alpelisib treatment (main objective). Patients will undergo a main evaluation visit with clinical, biological, functional and neurocognitive exams every 6 months. Safety monitoring will be assured on a monthly basis. The schedule and content of the visits are detailed in supplemental material.

Participants. Patients aged 2-40 years, with documented evidence of postzygotic or constitutional variant(s) in *PIK3CA* and a diagnosis of MCAP with a neurocognitive disorder (from specific learning disorder to severe intellectual disability) at the time of consent could be included. The main exclusion criteria are related to contra-indications to alpelisib treatment such as history of pancreatitis, diabetes, or pneumonitis.

Randomisation. Randomisation will be performed by the site staff using the centralized tool in the e-CRF at baseline visit, only after confirming that the participant fulfills all the inclusion/exclusion criteria. Investigator will have to confirm the key eligibility criteria checklist embedded in the system to access to randomization tool in the e-CRF. A statistician from Dijon Bourgogne University hospital, independent of the research, will edit the randomization list, prior to the start of the trial. Breaking of blinding can be requested by the

investigator for occurrence of serious adverse events requiring knowledge of the experimental product to determine the therapeutic course to be taken, by the unblinding function in the randomization tool in the e-CRF.

Objectives

Primary objective

The primary objective is to demonstrate the efficacy on adaptive behavior after 24 months of alpelisib treatment.

Secondary objectives

To assess: (1) the efficacy of alpelisib vs placebo on adaptive behavior based on the comparison of the proportion of participants with response at 6 months in each group, (2) the impact of alpelisib treatment on cerebral and spinal cord vascularization and volume, (3) the safety of alpelisib treatment.

Exploratory objectives

To evaluate the effects of alpelisib on: (1) adaptive behavior at 6 months, (2) quality of life and clinical global impression, (3) neuropsychological parameters, (4) epilepsy, (5) overgrowth and skin lesions when appropriate, (6) hypotonia, and (7) to quantify alpelisib passage throughout the blood-brain barrier and its relationship with systemic exposure of alpelisib.

Study Endpoints

Primary outcome

According to the publication by Chatham et al. (18), clinically meaningful improvement will be defined as a gain of at least 4 points in the Vineland II Adaptive Behavior Scale (VABS-II) at 24 months of treatment compared to baseline. The VABS-II is the most widely used scale to assess day-to-day adaptive skills, from birth to adulthood (19, 20). It consists of a form which will be filled during an interview with an adult who is familiar with the daily living activities of the patients (usually a parent). The VABS-II is organized within a three-domain structure: communication, daily living skills, and socialization. In addition, VABS-II has a motor skills domain for children younger than 6 years of age, and an optional maladaptive behavior index (19, 20). The domain (communication, daily living skills, and socialization) standard scores have a mean of 100 and a standard deviation of 15. Adaptive levels can also be determined. A global standard score can also be computed (the Adaptive Behavior Composite standard score) and also has a mean of 100 and a standard deviation of 15.

Secondary outcomes

For the secondary objectives, the corresponding outcomes will include:

(1) The response (yes/no) defined as an improvement of at least 4 points in the VABS-II at 6 months of treatment in the alpelisib group compared to the placebo group.

(2) The changes in brain volume, vascularization, structural connectivity, assessed by MRI, from baseline to end of treatment period.

(3) The number, type and severity of adverse events.

Exploratory outcomes

Exploratory endpoints will assess the following:

(1) Improvement of at least 4 points in the VABS-II at 12, and 18 months of treatment, compared to baseline.

(2) Evolution of quality-of-life questionnaires, scores at visual analogue scale, and evolution of Clinical Global Impression of severity and Global improvement scores at 6, 12, 18, 24 months of treatment, compared to baseline.

(3) Changes in neuropsychological scales, adapted to age, at 12 and 24 months of treatment compared to baseline for attention, cognition, visuo-spatial disorders, fine motor skills, speech, reasoning and cognitive inhibition abilities, and at 24 months of treatment compared to baseline for IQ scale.

(4) Description of changes in seizures frequency (weekly diary), and antiepileptic drugs use at 6, 12, 18 and 24 months of treatment compared to baseline.

(5) Changes in overgrowth or skin lesions, classified as follows: increase, no changes or reduction in overgrowth or skin lesions according to clinical measures and evaluation of standardized photographs taken at 6, 12, 18 and 24 months of treatment compared to baseline.

(6) Changes in MFM (Motor Function Measure) scores at 6, 12, 18 and 24 months of treatment compared to baseline.

(7) Level of alpelisib (ng/mL) in cerebrospinal fluid (CSF) and in blood at between 6 or 24 months of treatment, and correlation estimate (rho) between CSF and blood levels of alpelisib.

The versions of each scale or questionnaire according to age are detailed in figure 2.

Sample size calculation:

The assumptions are as follows: 1) when following untreated MCAP patients 6.0% at best may have experienced the 4 points improvement, 2) the minimal requirement for the treatment to be considered clinically relevant by regulatory bodies is at least one third of treated patients (33%) experiencing the 4 points improvement. With alpha = 0.05, 1-beta = 0.90 and a bilateral test, 16 patients are needed to prove statistical difference between the theoretical and observed proportion. We are thus planning to enroll and analyze 20 patients to take account of possible loss of follow-up or withdrawal of consent. The approach for sample size calculation is also pragmatic, based on known and estimated cohort. A cohort of about 60 patients with MCAP and *PIK3CA* pathogenic variant is available, two thirds of them having ID or learning disability that could justify being enrolled in a clinical trial. If a patient is withdrawn from the study before treatment initiation, he will be replaced, to be able to conduct the comparison between alpelisib and placebo groups with 10 patients in each group.

PATIENT AND PUBLIC INVOLVEMENT

The feasibility of recruitment is assured by the participation of experts in the clinical and molecular aspects of PROS, including the French reference center for mosaic disorders, the RHU COSY (21), and the French patient's association for MCAP (M-CM France).

DATA MANAGEMENT AND DATA ANALYSES

Data collection, monitoring and management

Clinical, biological and radiological data, will be entered directly into a dedicated e-CRF (electronic Case Report Form) on the CleanWEB platform by the investigators, helped by Clinical Research Associate (CRA). The patient diary is paper-based and will be reviewed by

the investigator to capture the safety events that will be entered in the e-CRF. Each patient is identified by a unique code including: the number of the recruiting center, the inclusion rank, the initials of the patient (first letter of surname and first name). Automatic queries due to missing and incoherent data after data entry can be immediately generated by the CleanWEB software. Requests for corrections may also be generated by the methodological support unit of CHU Dijon and sent to the local and/or the evaluation center. The corrections will be made directly in the e-CRF by the investigators, assisted by the CRAs. Histories of changes are systematically recorded. Additionally, a CRA will perform an on-site exhaustive data monitoring for all patients. A data management plan, specific to the study, was prepared before initiating the study in the participating centers.

Statistical Analyses

Descriptive analysis

Descriptive analysis with presentation of the baseline characteristics of the cohort, with qualitative variables expressed as number of events with their frequencies (%), with their 2-sided exact 95% confidence interval (CI). For quantitative variables, mean (±SD) or median (IQR) values will be calculated.

Primary outcome analysis

Fisher's exact test or chi square test will be used to compare the observed proportion of patients reaching the primary endpoint after 24 months of treatment to the theoretical proportion of 6%. A $p<0.05$ will be considered significant.

Secondary analyses

The same approach will be used to compare the proportion of responders at M6 between alpelisib and placebo groups. Comparison of brain volumes (affected and unaffected zones) at after 24 months of treatment versus baseline will be performed using a paired-t-test or Wilcoxon rank test according to the distribution priorly assessed by a Shapiro-Wilk test.

For all safety analyses, data recorded during monitoring for adverse events (AE), either clinical or biological, will be collated and the number, type grade of AE, and their relation to treatment, will be described and the frequencies of AEs recorded as percentages and 95% CI. Results will be presented for the overall 24-month-period of treatment, and also specifically for the double-blind period, to compare safety between alpelisib and placebo. The CTCAE (v5.0) classification will be used to grade the events.

Exploratory analyses

Comparison of the mean scores of neuropsychological scale scores obtained at baseline and M24 will be performed using a paired-t-test or Wilcoxon rank test. To test the reliability of the change, the Reliable Change Index (RCI) will be calculated for each psychometric scale (22).

For fine reasoning and cognitive inhibition abilities, the raw data (logfiles from Presentation software) will be analyzed automatically using Matlab 7.1. A Reaction Time (RT) and an Error Rate (ER) analysis will be performed using R software (see supplemental material).

Change in scores at the MFM test will be assessed by analyzing the slopes of change between scores obtained at 6, 12, 18 and 24 months of treatment vs. baseline for each patient, and expressed as an annual rate using the unweighted least-square estimate. Comparison of mean scores will then be performed using a paired-t-test or Wilcoxon rank test.

Finally, the correlation between CSF level and blood level of alpelisib will be estimated by calculating the factor rho of Spearman's correlation.

Statistical Software

Analysis will be performed using SAS software (version 9.4). Statisticians will be blinded to the study.

METHODS: MONITORING

Harms: Steering data and safety monitoring committees

The coordinating centre at University Hospital Dijon-Bourgogne, Clinical Investigation Centre (CIC INSERM 1432), is assigned the responsibility of all study aspects: ethical, regulatory, study coordination, data management and publication strategy.

The steering committee is composed by the coordinating investigator, a methodologist, a pharmacovigilance officer, a pharmacist, a sponsor representative, and the project manager. This committee meets on a weekly basis to assess study progress and solve potential issues.

An independent data safety monitoring board (DSMB) is monitoring the patients' safety during the study and gives recommendations to the steering committee. It is composed of a pharmacologist or pharmacovigilant officer, a metholologist, a neuropediatrician, and a geneticist. The DSMB met when the first 4 included patients (25% of the initially anticipated number of patients) had completed 1 month of treatment, and will meet again at 50% and 75% of the inclusions. Treatment initiations will be halted at each threshold and will resume after review of the safety data by the DSMB.

ETHICS AND DISSEMINATION

Authorization was obtained from the French National Drug Safety Agency on 28/07/2022 and from the Ethics Committee (CPP Sud-Ouest et Outre-Mer I) on 22/09/2022 (reference number: 2022-500197-34-01). The protocol was registered with ClinicalTrials.gov under the identifier NCT05577754 in 13/10/2022. Current version of protocol is V3 (25/05/2023). The first patient was included in November 2022 and the study is expected to be completed by 2026.

Results will be presented at scientific meetings and published in international peer-reviewed journals.

DISCUSSION

At present, there is no licensed or unlicensed drug with a proven benefit for patients with MCAP syndrome, although alpelisib FDA's approval for patients with PROS, based on collection of real-world evidence from compassionate use, makes it possible to treat patients with MCAP syndrome. As such, there is a clear unmet medical need. The understanding of the pathophysiology of the PROS and MCAP syndromes, a *PIK3CA* gain-of-function variant, and the development of specific PI3K3 inhibitors in cancer, where the same variants are found, has raised great hopes of eventually providing an effective treatment.

Whereas Novartis Pharmaceutical's sponsored EPIK-P2 is running for patients with PROS, no such study was planned for patients with MCAP syndrome. Even if the data are sparse, Venot et al. reported that the two patients with MCAP syndrome among the 19 patients with PROS syndrome, exhibited improvement in cognitive function, behavior, and cerebral perfusion (15). We therefore hypothesized that alpelisib treatment could be useful for these patients and designed a dedicated trail.

The judgement criteria for the SESAM study were based on the collection of complete clinical and radiological data from 33 French patients, which we published in 2021 (10). It was this cohort that led us, for example, not to adopt the evolution of epileptic seizures as the primary endpoint, as only 10% to 15% of patients suffer from them.

We chose VABS as the primary endpoint, as it has been evaluated in clinical trials on neurodevelopmental disorders, to assess the changes experienced by patients/families in their daily lives (18). Nevertheless, in the absence of national or international consensus on the scales to be used in clinical trials aimed at demonstrating an improvement in patients with neurocognitive disorders, we have chosen to use a battery of scales, all addressing complementary domains, appropriate to the age, cognitive level, ability of the patients to concentrate, and the time constraints between two assessments. We believe that the results of this trial will provide useful information for future clinical trials targeting neurodevelopmental pathologies. We added a simplified paradigm matrix stimuli especially designed by the expert team to try to identify fine points of improvement in clinical trials that would be difficult to demonstrate with conventional scales (23). Although we have no certainty about the possibility of achieving a reduction in brain volume, we felt it is important to assess this. Therefore, volumetric MRI will be performed at the beginning of the study and at the end of the treatment. Given the poor knowledge of the prevalence of spinal cord abnormalities in MCAP, spinal cord MRI was added to the protocol. Assessing quality of life as well as the effect of alpelisib on other non-brain manifestations of the disease were also planned.

The passage of alpelisib through the blood-brain barrier has only been hypothesized, based on the observation of a reduction in the size of brain metastases in women with breast cancer or an improvement in epileptic seizures. It is therefore necessary to assess and quantify the

passage of alpelisib into the cerebrospinal fluid (CSF), in relation with the plasmatic dosage. This will help to establish a correlation between the plasma concentration and that of the CSF, possibly allowing therapeutic monitoring on the basis of plasma concentrations alone (24).

The SESAM trial also benefits from the accumulated experience of our previous trials, one with mTOR inhibitor sirolimus, and the other with taselisib, another PI3Kα-specific inhibitor (25, 26). In particular, conducting this trial as part of a well-established network with investigators from local centres, enabling some of the follow-up visits to be carried out close to patients' homes, improves acceptability and ensures that the trial runs smoothly.

In addition, by using this two-period design which allows all patients to be treated, we aim to demonstrate that a rigorous methodology can be applied in clinical trials in rare diseases, with high quality standards while preserving the acceptability of the trial's burden for the patient. Based on our preliminary data in CLOVES, we will enhance knowledge relating to the efficacy and side-effect profile associated with long-term treatment with *PI3K* pathway inhibition, and determine the effectiveness of treatment across an expanded number of PROS. Considering the pathological overlap deriving from the common feature of *PIK3CA* upregulation, our work may also inform future transversal therapeutic strategies in the context of a larger group of diseases.

DATASHARING

All requests for the study's data will be considered by the SESAM trial steering committee.

TRIAL STATUS

Recruitment is ongoing (16 patients included as of 31/12/2023).

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REFERENCES

1. Lindhurst MJ, Parker VE, Payne F, Sapp JC, Rudge S, Harris J, et al. Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA. Nat Genet. 2012;44(8):928-33.

2. Rivière JB, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, et al. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet. 2012;44(8):934-40.

3. Kurek KC, Luks VL, Ayturk UM, Alomari AI, Fishman SJ, Spencer SA, et al. Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. Am J Hum Genet. 2012;90(6):1108-15.

4. Rios JJ, Paria N, Burns DK, Israel BA, Cornelia R, Wise CA, et al. Somatic gain-of-function mutations in PIK3CA in patients with macrodactyly. Hum Mol Genet. 2013;22(3):444-51.

5. Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. Oncogene. 2008;27(41):5486-96.

6. Kuentz P, St-Onge J, Duffourd Y, Courcet JB, Carmignac V, Jouan T, et al. Molecular diagnosis of PIK3CA-related overgrowth spectrum (PROS) in 162 patients and recommendations for genetic testing. Genet Med. 2017;19(9):989-97.

7. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, Parker VE, Blumhorst C, Darling T, et al. Clinical delineation and natural history of the PIK3CA-related overgrowth spectrum. Am J Med Genet A. 2014;164a(7):1713-33.

8. Keppler-Noreuil KM, Rios JJ, Parker VE, Semple RK, Lindhurst MJ, Sapp JC, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am J Med Genet A. 2015;167a(2):287-95.

9. Mirzaa GM, Rivière JB, Dobyns WB. Megalencephaly syndromes and activating mutations in the PI3K-AKT pathway: MPPH and MCAP. Am J Med Genet C Semin Med Genet. 2013;163c(2):122-30.

10. Garde A, Guibaud L, Goldenberg A, Petit F, Dard R, Roume J, et al. Clinical and neuroimaging findings in 33 patients with MCAP syndrome: A survey to evaluate relevant endpoints for future clinical trials. Clin Genet. 2021;99(5):650-61.

11. Haute Autorité de Santé Procotole; National de Diagnostic et de Soins for MCAP [Available from: https://www.has-sante.fr/upload/docs/application/pdf/2021-10/pnds-mcap_sept_2021.pdf.

12. Douzgou S, Rawson M, Baselga E, Danielpour M, Faivre L, Kashanian A, et al. A standard of care for individuals with PIK3CA-related disorders: An international expert consensus statement. Clin Genet. 2022;101(1):32-47.

13. Arafeh R, Samuels Y. PIK3CA in cancer: The past 30 years. Semin Cancer Biol. 2019;59:36-49.

14. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2019;380(20):1929-40.

15. Venot Q, Blanc T, Rabia SH, Berteloot L, Ladraa S, Duong JP, et al. Targeted therapy in patients with PIK3CA-related overgrowth syndrome. Nature. 2018;558(7711):540-6.

16. Food and Drug Administration - FDA approves alpelisib for PIK3CA-related overgrowth spectrum 2022 [updated 04/06/2022. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-alpelisib-pik3ca-related-overgrowth-spectrum.

17. Morin G, Degrugillier-Chopinet C, Vincent M, Fraissenon A, Aubert H, Chapelle C, et al. Treatment of two infants with PIK3CA-related overgrowth spectrum by alpelisib. J Exp Med. 2022;219(3).

18. Chatham CH, Taylor KI, Charman T, Liogier D'ardhuy X, Eule E, Fedele A, et al. Adaptive behavior in autism: Minimal clinically important differences on the Vineland-II. Autism Res. 2018;11(2):270-83.

19. Sparrow SS, Balla, D. A., & Cicchetti, D. V. . Vineland adaptive behavior scales: Interview edition, survey form manual. Circle Pines, MN: American Guidance Service. 1984.

20. Sparrow SSC, D.V.; Saulnier, C.A. Vineland-3: Vineland Adaptive Behavior Scales. 3rd ed. Pearson Assessments; Minneapolis, MN, USA. 2016.

21. Recherche Hospitalo Universitaire (RHU) - Cure Overgrowth Syndromes (COSY) [Available from: https://rhu-cosy.com/.

22. Jacobson NS, Truax P. Clinical significance: a statistical approach to defining meaningful change in psychotherapy research. J Consult Clin Psychol. 1991;59(1):12-9.

23. Curie A, Brun A, Cheylus A, Reboul A, Nazir T, Bussy G, et al. A Novel Analog Reasoning Paradigm: New Insights in Intellectually Disabled Patients. PLoS One. 2016;11(2):e0149717.

24. Batalini F, Moulder SL, Winer EP, Rugo HS, Lin NU, Wulf GM. Response of Brain Metastases From PIK3CA-Mutant Breast Cancer to Alpelisib. JCO Precis Oncol. 2020;4.

25. Parker VER, Keppler-Noreuil KM, Faivre L, Luu M, Oden NL, De Silva L, et al. Safety and efficacy of low-dose sirolimus in the PIK3CA-related overgrowth spectrum. Genet Med. 2019;21(5):1189-98.

26. Luu M, Vabres P, Devilliers H, Loffroy R, Phan A, Martin L, et al. Safety and efficacy of low-dose PI3K inhibitor taselisib in adult patients with CLOVES and Klippel-Trenaunay syndrome (KTS): the TOTEM trial, a phase 1/2 multicenter, open-label, single-arm study. Genet Med. 2021;23(12):2433-42.

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AUTHORS' CONTRIBUTIONS :

LF and VB initiated this study. LF, ML, MB designed the study. ML has written this manuscript. LF, MB, AM, MC (sponsor), and Novartis Pharmaceuticals (sponsor) reviewed the manuscript. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

A patent application ("BYL719 (alpelisib) for use in the treatment of PIK3CA-related overgrowth spectrum" #WO2017140828A1) has been filed by INSERM (Institut National de la Santé et de la Recherche Médicale), Centre National De La Recherche Scientifique (CNRS), Université Paris Cité, and Assistance Publique-Hôpitaux De Paris (AP-HP) for the use of BYL719

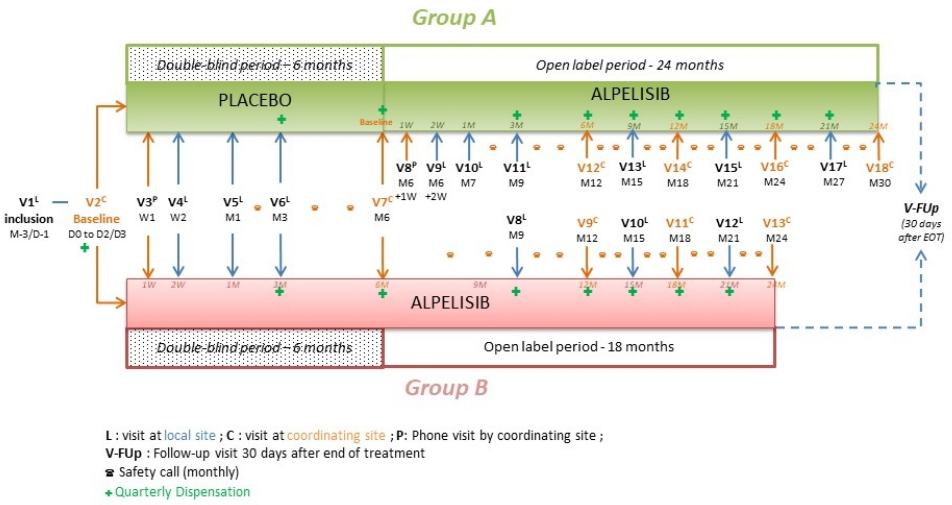
(alpelisib) in the treatment of PIK3CA-related overgrowth spectrum (PROS/CLOVES syndrome). Dr. Canaud is the inventor. This patent is licensed to Novartis Pharmaceutical.

ML has received consulting fees from Novartis Pharmaceutical. GC receives or has received consulting fees from Novartis Pharmaceutical, Fresenius Medical Care, Vaderis, Alkermes, IPSEN and BridgeBio.

LEGENDS

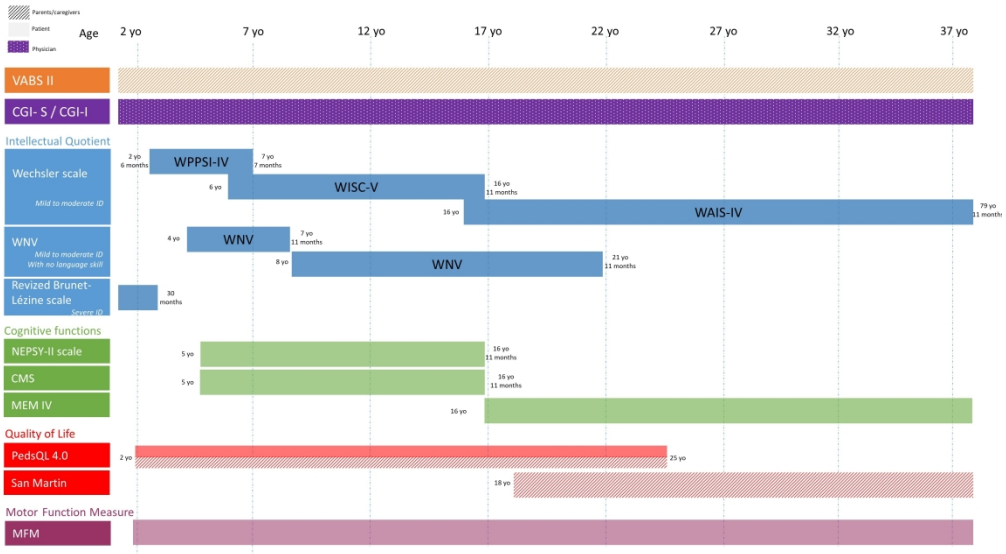
Figure 1. Study design of the SESAM trial

Figure 2. Neuropsychological tests used in the SESAM trials. Tests were selected according to the conclusions of a working group from the DéfiScience Network (www.defiscience.fr), which assessed all the neuropsychological tests available for each domain and adapted to ID patients. The 20-item Motor Function Measure (MFM20) will be administered for children <7 yo, and the MFM32 for children ≥7yo.



Study design of the SESAM trial

254x190mm (96 x 96 DPI)



Neuropsychological tests used in the SESAM trials. Tests were selected according to the conclusions of a working group from the DéfiScience Network (www.defiscience.fr), which assessed all the neuropsychological tests available for each domain and adapted to ID patients. The 20-item Motor Function Measure (MFM20) will be administered for children <7 yo, and the MFM32 for children ≥7yo.

338x190mm (300 x 300 DPI)

SUPPLEMENTAL MATERIAL

Annex 1. Complete list of inclusion and exclusion criteria

1. Signed informed consent and assent (when applicable) from the patient, parent, or guardian must be obtained prior to any study related screening procedures are performed.
2. Male or female patients age ≥2 years and ≤40 years at the time of informed consent
3. Patients with diagnosis of MCAP* with neurodevelopmental disorder presentation (from specific learning disorder to severe intellectual disability)
   The most recent set of diagnostic criteria for MCAP includes five core features: progressive megalencephaly (criterion 1), developmental vascular disorders (criterion 2), distal limb anomalies (criterion 3), cortical brain malformations (criterion 4), connective tissue dysplasia (criterion 5) plus supportive features. MCAP syndrome is diagnosed in the presence of criterion 1 plus either criterion 2 or criterion 3 (Mirzaa et al., 2013). The absence of criterion 2 and 3 can be accepted in constitutional variant.
4. Documented evidence of a somatic or constitutional mutation(s) in the PIK3CA gene performed in local laboratories using a Deoxyribonucleic acid (DNA) based validated test at the time of informed consent.
5. Adequate bone marrow and organ function (assessed during the screening visit):
   a. Absolute neutrophil count ≥ 1.5 × 109/L
   b. Platelets ≥ 100 × 109/L
   c. Hemoglobin ≥ 9.0 g/dL (transfusions are allowed)
   d. Calcium (corrected for serum albumin) and magnesium within normal limits or ≤Grade 1 according to NCI-CTCAE version 5.0 if judged clinically not significant by the investigator
   e. Potassium within normal limits.
   f. INR ≤1.5
   g. Creatinine Clearance ≥ 30 mL/min using Modification of Diet in Renal Disease (MDRD) (≥18 years old) or creatinine-based Bedside Schwartz (<18 years old) Glomerular filtration rate (GFR) equation
   h. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN.
   i. Total bilirubin< ULN except for patients with Gilbert's syndrome who may only be
   j. Fasting plasma glucose (FPG) ≤ 140 mg/dL (7.7 mmol/L) and Glycosylated hemoglobin (HbA1c) ≤ 6.5% (both criteria have to be met)
   k. Fasting Serum amylase ≤ 2 × ULN
   l. Fasting Serum lipase ≤ ULN
6. Able to swallow study drug according to age: tablets, or as drinkable suspension, or granules (under development)
7. For women of child-bearing potential only: negative pregnancy test at screening visit
8. Male patients with sexual partners who are pregnant, possibly pregnant or who could become pregnant should use condoms during sexual intercourse for the duration of the study and for one week following discontinuation of alpelisib.
9. For exploratory study only: signed informed optional consent for lumbar puncture

EXCLUSION CRITERIA

Participants meeting any of the following criteria are not eligible for inclusion in this study:

1. Patient previously treated with alpelisib
2. Known impairment of GI function due to concomitant disease that may significantly alter the absorption of the study drug (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection) at time of informed consent.
3. Participant with uncontrolled diabetes mellitus (Type I or II) at time of informed consent.
4. History of hypersensitivity to any drugs or metabolites of PI3K inhibitor or any of the excipients of alpelisib at time of informed consent.
5. Participant with other concurrent severe and/or uncontrolled medical conditions that would, in the treating Physician's judgment, contraindicate administration of alpelisib (e.g., active and/or uncontrolled severe infection, chronic active hepatitis, hepatic impairment Child Pugh score C, immuno-compromised, etc.) at time of informed consent.

6. Female participants of childbearing potential and male participants who do not agree at time of informed consent to abstinence or, if sexually active, unwilling to use a condom and/or a highly effective method of contraception for the duration of the study and for one week following discontinuation of alpelisib. Highly effective contraception methods is one of the following:
    a. Total abstinence: when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
    b. Female sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or bilateral tubal ligation at least six weeks before taking alpelisib. In case of oophorectomy alone, only when the reproductive status of the female has been confirmed by follow-up hormone level assessment
    c. Male sterilization at least 6 months prior to screening. The vasectomized male partner should be the sole partner for that study participant d. Use of oral, injected or implanted hormonal methods of contraception or placement of an intrauterine device or intrauterine system or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception

   If local regulations deviate from the contraception methods listed above to prevent pregnancy, local regulations apply and will be described in the ICF.
7. Treatment by any mTOR or PI3K-AKT signaling pathway inhibitor within 1 monthbefore inclusion
8. History of prior and or ongoing malignancy (within 5 years before informed consent except radically treated Carcinoma in situ of radically treated basal-cell carcinoma of skin or thyroid gland well differentiated microcarcinoma or Stage 1 Wilms' tumor of a histology other than anaplastic), or ongoing investigations or treatment for malignancy at time of informed consent.
9. Treatment with strong inducers of CYP3A4 and inhibitors of Breast Cancer Resistance Protein (BCRP) that cannot be stopped at least the week prior to the screening
10. Debulking or other major surgery performed within 3 months at time of informed consent
11. Known history of Steven Johnson's syndrome, erythema multiform or toxic epidermal necrolysis at time of informed consent.
12. For participants ≥ 6 years of age: Participants with documented pneumonitis or interstitial lung disease at the time of informed consent and with impaired lung function (e.g., FEV1 (Forced expiratory volume) or DLCO (Diffusing Capacity of the Lung for Carbon Monoxide) ≤ 70% of predicted) that is not related to PROS.
13. For participants between 2 to 5 years of age: Participants with documented or suspicious pneumonitis or interstitial lung disease based on MRI images at time of informed consent.
14. History of acute pancreatitis within 1 year before informed consent or past medical history of chronic pancreatitis at time of informed consent.
15. Clinically significant heart disease at time of informed consent, including:
    a. History of documented congestive heart failure (New York Heart Association functional classification III-IV)
    b. Clinically significant uncontrolled cardiac arrhythmias
    c. Long QT syndrome, family history of idiopathic sudden death or congenital long QTsyndrome
    d. Corrected QT (QTcF) at screening: >470 ms for ≥18 years old / >450 ms for <18 years old
    e. Creatinine clearance < 70ml/min/1.73 m²
16. Patient currently, or in the 3 months before inclusion, enrolled in another interventional trial.
17. Person not affiliated to a national health insurance scheme
18. Patient, parents or legal authorized representative incapable of expressing consent
19. Inability to attend all trial visits
20. For the optional lumbar puncture only : known intracranial hypertension, active infection at puncture site, known coagulation disorders, Platelets < 50 × 10^9/L

Annex 2a. Assessment Schedule for Group A : patients allocated to placebo during double-blind period

Period		Screening	Double-blind period : PLACEBO treatment						Open-label period: ALPELISIB treatment											F Up
Study duration		max 3 m	D0	W1	W2	M 1	M 3	M 6	M 6+1W	M 6+2W	M 7	M 9	M 12	M 15	M 18	M 21	M 24	M 27	M 30	M 31
treatment duration									1w	2w	1m	3 m	6 m		12 m		18 m		24 m	
Visit Name	Visit 0 Information	Visit 1 Inclusion	Visit 2 Baseline	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7 Interim evaluation Baseline	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 Interim evaluation	Visit 13	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18 End of treatment (EOT)	Visit F Up follow -up
Week	<W-12	W-12 to D-1	D0 to D2 or D3	W1	W2	W4	W12	W24	W25	W27	W28	W36	W52		W72		W104			30 days after EOT
Site	Assessment	Local	Assessment	Phone call	Local	Local	Local	Assessment	Phone call	Local	Local	Local	Assessment	Local	Assessment	Local	Assessment	Local	Assessment	Local
Window s allow ed				± 1 days	± 2 days	± 7 days	± 7 days	± 7 days	± 1 days	± 2 days	± 7 days	± 7 days	± 7 days	± 14 days	± 14 days	± 14 days	± 14 days	± 14 days	± 14 days	± 3 days
Informed Consent	Information	●																		
Inclusion/exclusion criteria	●	●																		
Demography		●																		
Medical and surgical history		●																		
Non drug therapies/surgical /medical procedures		●	Continuous up to 30 days after the last dose of study treatment																	
Concomitant medications		●	Continuous up to 30 days after the last dose of study treatment																	
Physical examination		●	●		●	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Vital Signs		●	●		●	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Visual Analogue Scale			●					●									●		●	
Electrocardiogram (EKG)		●	●		●	●		●		●	●				●		●		●	
Digital Photography of affected areas			●					●							●		●		●	
Measurements of hypertrophic areas			●					●											●	
Brain MRI			●																●	
Spinal cord MRI ♦			●																●	
Blood sample		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
HbA1C		●					●	●				●		●	●		●		●	●
Hepatitis and HIV		●																		
Pregnancy test*		●	●			●	●	●			●	●	●	●	●	●	●	●	●	●
One optional lumbar puncture + blood sample for alpelisib dosages between 6 to 24months of treatment	optional consent														●		●		●	
VABS-II			●					●							●		●		●	
Wechsler scale and profil** /***			●																●	
Revised Brunet-Lézine scale ***			●																●	
NEPSY-II ***			●												●				●	
CMS/MEM***			●												●				●	
CGI-S and CGI-I			CGI-S					●							●		●		●	
Fine reasoning and inhibition abilities			●					●							●		●		●	
QoLquestionnaire ***			●					●							●		●		●	
MFM scale			●					●							●		●		●	
Randomisation			●																	
Study Drug Dispensation			●				●	●				●	●	●	●	●	●	●		
Monitoring post first administration			● during 30mins					● during 30mins												
Education of guardians on clinical signs of alpelisib toxicity/overdos			●					●												
Unblinding data								● post evaluation												
Epilepsiy daily diary			daily diary completed by patient																	
Medical diary daily dosing and any AE experienced			daily diary completed by patient																	
Adverse events		●†	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Annex 2b. Assessment Schedule for Group B : patients allocated to alpelisib during double-blind period

Period		Screening	Double-blind period : ALBELISIB treatment						Open-label period: ALPELISIB treatment						
Study duration /patient		max 3 m	D0	W1	W2	M 1	M 3	M 6	M 9	M 12	M 15	M 18	M 21	M 24	M 25
treatment duration				1w	2w	1m	3 m	6 m		12 m	15m	18m	21m	24m	
Visit Name	Visit 0 Information	Visit 1 **Inclusion**	Visit 2 **Baseline**	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7 **Interim evaluation**	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13 **End of treatment (EOT)**	Visit F Up follow -up
Week	<W-12	W-12 to D-1	D0 to D2 or D3	W1	W2	W4	W12	W24		W52				W104	30 days after EOT
Site	Assessment	Local	Assessment	Phone call	Local	Local	Local	Assessment	Local	Assessment	Local	Assessment	Local	Assessment	Local
Windows allowed				± 1 days	± 2 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days	± 14 days	± 14 days	± 14 days	± 14 days	± 3 days
Informed Consent	Information	●													
Inclusion/exclusion criteria	●	●													
Demography		●													
Medical and surgical history		●													
Non drug therapies/surgical/medical procedures		●	Continuous up to 30 days after the last dose of study treatment												
Concomitant medications		●	Continuous up to 30 days after the last dose of study treatment												
Physical examination		●	●		●	●	●	●	●	●	●	●	●	●	●
Vital Signs		●	●		●	●	●	●	●	●	●	●	●	●	●
Visual Analogue Scale			●					●		●		●		●	
Electrocardiogram (EKG)		●	●		●	●		●		●		●		●	
Digital Photography of affected areas			●					●		●		●		●	
Measurements of hypertrophic areas			●					●						●	
Brain MRI			●											●	
Spinal cord MRI ♦			●											●	
Blood sample		●	●	●	●	●	●	●	●	●	●	●	●	●	●
HbA1C		●					●	●	●	●		●		●	●
Hepatitis and HIV		●													
Pregnancy test*		●	●			●	●	●	●	●	●	●	●	●	●
One optional lumbar puncture + blood sample for alpelisib dosages between 6 to 24months of treatment	optional consent							●		●		●		●	
VABS-II			●					●		●		●		●	
Wechsler scale and profil** /***			●											●	
Revised Brunet-Lézine scale ***			●											●	
NEPSY-II ***			●							●				●	
CMS/MEM***			●							●				●	
CGI-S and CGI-I			CGI-S					●		●		●		●	
Fine reasoning and inhibition abilities			●					●		●		●		●	
QoLquestionnaire ***			●					●		●		●		●	
MFM scale			●					●		●		●		●	
Randomisation			●												
Study Drug Dispensation			●				●	●	●	●	●	●	●		
Monitoring post first administration			●during 30 min												
Education of guardians on clinical signs of lithium toxicity/overdos			●					●							
Unblinding data								●post evaluation							
Epilepsiy daily diary			daily diary completed by patient												
Medical diary daily dosing and any AE experienced			daily diary completed by patient												
Adverse events		●†	●	●	●	●	●	●	●	●	●	●	●	●	●

MRI : Magnetic Resonance Imagery ; VABS-II: Vineland Adaptive Behaviour Scales second edition; HbA1C: Glycated hemoglobin; HIV: human immunodeficiency virus; NEPSY-II: A Developmental NEuroPSYchological Assessment – second edition; CMS: Children Memory Scale; CGI-S: Clinical Global Impressions Scale - Severity of illness; CGI-I : Clinical Global Impressions Scale - improvement scale; MFM : Motor Function Measure

A spinal cord sequence will be offered in parallel. It would be optional if the affected individual could not stay in the MRI for a sufficient time and if anomalies detected during the 1st imaging

* Pregnancy test can be urinary or blood, except at baseline, EoT and FuP, where blood sample is mandatory

** Wechsler scale according to patient age: WPPSI-IV (2 years et 6 months to 7 years et 7 months); or WISC-V (6 years to 16 years 11 months); or WAIS-IV (16 years to 79 years 11 months); Wechsler Nonverbal Scale of Ability (WNV)

*** adapted to age and level of ID (see schema 2)

† Only if suspected to be linked with the study procedures

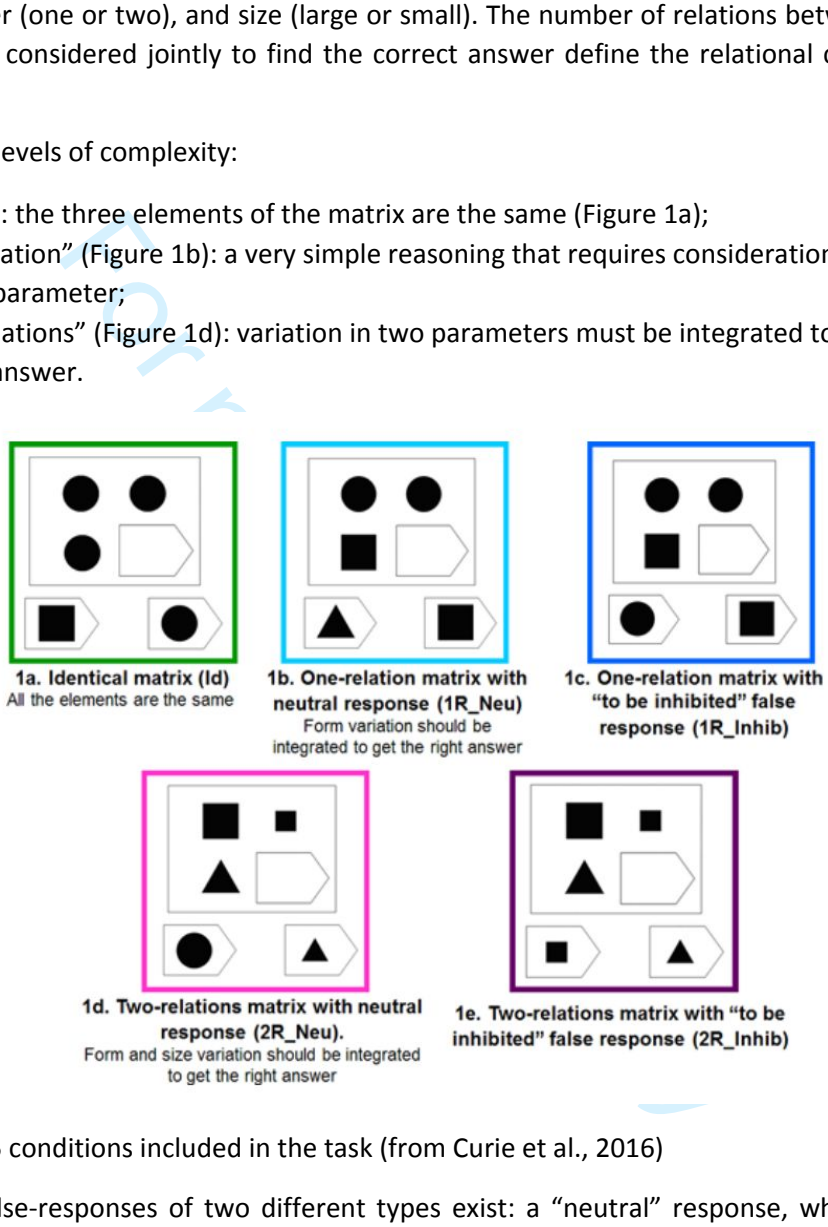
Annex 3. Computer task for visual analogical reasoning paradigm

Simple Matrices task

The simplified paradigm matrix stimuli especially designed by the expert team consist of four elements (instead of the nine cells in Raven's), with two response choices (instead of the eight in Raven's). Four parameters are involved in matrix creation: color (white, black or grey), form (round, square or triangle), number (one or two), and size (large or small). The number of relations between the items that need to be considered jointly to find the correct answer define the relational complexity of a matrix.

There are three levels of complexity:

- Identical: the three elements of the matrix are the same (Figure 1a);
- "One-relation" (Figure 1b): a very simple reasoning that requires consideration of only one varying parameter;
- "Two-relations" (Figure 1d): variation in two parameters must be integrated to get the correct answer.



Example of the 5 conditions included in the task (from Curie et al., 2016)

Furthermore, false-responses of two different types exist: a "neutral" response, which is a choice different from the items displayed in the matrix (Figure 1b and 1d) and a "to be inhibited" response, which is identical to one of the matrix items displayed (Figure 1c and 1e). Thus, this task combines 5 different conditions: identical matrices (Id), one-relation matrices with neutral responses (1R_Neu), onerelation matrices with to be inhibited responses (1R_Inhib), two-relations matrices with neutral responses (2R_Neu) and two-relations matrices with to be inhibited responses (2R_Inhib).

Task Description/data acquisition

The participants are asked to identify the missing element that completes a pattern. The stimuli are displayed on a computer screen. Participants are told to find the best answer to fill in the missing "piece". For the patients, immediate visual feedback via a happy or sad emoticon (in case of right or

wrong answer, respectively) is given during the training to make the task easier to understand. The paradigm will consist of four runs. Each matrix is preceded by a fixation cross. The subject then has to select the correct answer by pushing a button. The number of each type of condition will be counterbalanced on each run, producing the same number of right and left answers. For each type of matrix, the type of variable relation (form, color, size, number) will also be counterbalanced. The order of different conditions in the matrix display will be randomized, as is the side of the correct answer. Each run consists of 45 trials in the behavioral task and 15 trials in the eyetracking task. The matrices are generated and displayed using Presentation software (http://www.neurobs.com). A break will be allowed if necessary between two runs.

BMJ Open

A Phase II double-blind multi-center, placebo-controlled trial, to assess the efficacy and safety of alpelisib (BYL719) in pediatric and adult patients with Megalencephaly-CApillary malformation Polymicrogyria syndrome (MCAP) – the SESAM study protocol

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A Phase II double-blind multi-center, placebo-controlled trial, to assess the efficacy and safety of alpelisib (BYL719) in pediatric and adult patients with Megalencephaly-CApillary malformation Polymicrogyria syndrome (MCAP) – the SESAM study protocol

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Abstract:

Introduction

The MCAP syndrome (Megalencephaly Capillary malformation Polymicrogyria) results from mosaic gain-of-function *PIK3CA* variants. Main clinical features are macrocephaly, somatic overgrowth, neurodevelopmental delay, and brain anomalies. Alpelisib (Vijoice®) is a recently FDA-approved PI3Kα-specific inhibitor for patients with PIK3CA-related overgrowth spectrum (PROS). During its development, in patients with MCAP subgroup of PROS, there was no specific, standardised evaluation of the effect on neuro-cognitive functioning. Moreover, it remains unknown if the molecule crosses the blood-brain barrier. Our objective is to evaluate the efficacy of a 24-month treatment with alpelisib on adaptive behaviour in patients with MCAP syndrome.

Methods and Analysis

SESAM is an industry-sponsored two periods multi-centre French academic phase II trial, with a 6 months double-blind, placebo-controlled period followed by open label period. Primary endpoint is a ≥4 points improvement in the Vineland II Adaptive Behavior Scale (VABS), 24 months after treatment initiation. Secondary objectives are safety, VABS improvement at 6 months, impact on quality of life, epilepsy and hypotonia. Twenty patients aged 2 to 40 years old, with a MCAP diagnosis and neurodevelopmental disorders of various degrees, will be followed monthly in local centres, centrally assessed (clinical, biological, neuropsychological and functional evaluation) at baseline and every 6 months. Patients will be evaluated by volumetric MRI at baseline and at 24 months. An optional lumbar puncture will be performed to investigate blood-brain barrier crossing. Inclusions were completed by April 2024, with the end of follow-up in November 2026.

Given the efficacy of alpelisib in patients with PROS, if the drug crosses the blood-brain barrier, we can expect a clinical benefit for patients with neurocognitive disorders.

Ethics and dissemination

Ethical approval was given by CPP Sud-Ouest et Outre-Mer I (reference: 2022-500197-34-01). Findings from this study will be disseminated via publication, reports and conference presentations.

Trial registration Number (ClinicalTrials.gov)

NCT05577754

Keywords:

Neurodevelopmental disorders, alpelisib, therapeutic trial, PIK3CA, MCAP

Word count: 3822 words

Strengths and limitations of this study:

- The SESAM trial is the first evaluation of alpelisib dedicated in patients with MCAP using neurocognitive endpoints.
- The passage of alpelisib through the blood-brain barrier will be evaluated in the SESAM trial.
- The two-period design (double blind placebo-controlled period followed by open-label period) has been chosen to comply with the best quality methodological standards.
- Heterogeneity in patient's clinical presentation represents a challenge in the interpretation of the results.
- No international consensus exists on the scales to be used in clinical trials in patients with neurocognitive disorders.

INTRODUCTION

Segmental overgrowth disorders (SODs) are rare conditions usually characterized by abnormal and asymetric growth of some parts of the body. This excessive tissue growth is caused by an overactivation of the cell proliferation mechanism. Mosaic activating variants in the p110α catalytic subunit of phosphatidylinositol-3 kinase (PI3K; encoded by the *PIK3CA* gene) have been identified in a subset of SODs. The PI3K-AKT-mTOR is a critical signalling pathway in regulating proliferation, survival and cell growth. Activating variants in *PIK3CA* lead to increased PI3K-AKT-mTORC1 axis activation, which in turn promotes excessive growth in affected tissues [1-6].

The PIK3CA-related overgrowth spectrum (PROS) is a congenital condition with progressively asymmetric overgrowth which can begin in the antenatal period. This disease is wide-ranging, and depends upon the timing of the founder mutation in embryogenesis [6-9]. Depending on the clinical presentation (segmental body overgrowth or brain disorder), three main disease subgroups can be distinguished: the CLOVES syndrome (Congenital Lipomatous Overgrowth, Vascular malformations, Epidermal nevi, Scoliosis/Skeletal/Spinal anomalies), the Klippel-Trenaunay syndrome and the MCAP syndrome (Megalencephaly Capillary malformation Polymicrogyria). CLOVES clinical presentation ranges from isolated digit enlargement to extensive overgrowth of limbs, thorax/abdomen and/or face. It may be accompanied by vascular or lymphatic malformations, epidermal nevi and spinal anomalies. Associated morbidity can be highly variable, but can include functional impairment, debilitating haemorrhages and thromboses, and, in some cases, can be lethal. For patients with Klippel-Trenaunay syndrome, vascular malformations are often in the foreground, associated with soft tissue and bone hypertrophy, and involve one limb, most often the lower limb. MCAP is

characterized by megalencephaly (large head), capillary malformation of the skin (middle face, limbs and trunk), abnormalities of the extremities and possible abnormalities of the brain structure (Chiari malformation, hydrocephalus, polymicrogyria) [9, 10]. Megalencephaly is present at birth, and the brain continues to develop gradually during the first postnatal years with a relative stabilization with age, although remaining larger than normal. MCAP patients may experience impaired cognition, hypotonia, variable intellectual deficiency and seizures. Abnormalities of the brain structure may be present (hydrocephalus, Chiari malformation and polymicrogyria in particular). Asymmetry of some body parts may also exist, as well as skin manifestations. Cardiac and genitourinary abnormalities have been reported in rare cases. The current diagnosis is established through a clinical evaluation and genetic testing on the affected tissues. In rare cases, the *PIK3CA* variant is present in all cells and not at a mosaic state. Magnetic resonance imaging (MRI) is used to identify and monitor brain abnormalities. The French National Protocol for Diagnosis and Care for MCAP syndrome [11] and an international expert consensus statement for standardizing care for individuals with *PIK3CA*-related disorders have been published [12]. As patients may be misdiagnosed, the true prevalence of the disease is not well known. But in France, in 2021, more than 60 MCAP patients had already been genotyped. Variability in the degree of neurocognitive manifestations is considerable, ranging from mild learning disabilities to profound intellectual disability, in some cases associated with epilepsy {10].

The natural history of PROS shows that most of the overgrowth progression occurs during early childhood, emphasizing the need to assess the potential benefit of early treatment in paediatric patients with PROS. It could avert associated complications and/or surgery by decreasing disease progression at this active stage.

Interestingly, drug treatments that specifically inhibit the p110α catalytic subunit of PI3K have been developed in oncology in case of tumors with *PIK3CA* gain-of-function variants. Alpelisib (Vijoice®, Novartis Pharmaceutical) have been authorized in metastatic breast cancer [13, 14], and has therefore been investigated through drug repurposing in PROS, in a case series of 19 French patients under a compassionate approach, including 2 with MCAP [15]. After encouraging results in safety and efficacy, Novartis Pharmaceutical started the EPIK program. The EPIK-P1 (NCT04285723) is a real-world study for demonstrating clinical benefit in people with PROS, and EPIK-P3 (NCT04980833) a phase II study to assess long-term safety and efficacy of alpelisib in people with PROS who participated in EPIK-P1. The ongoing international prospective phase II double-blind, randomized, placebo-controlled study clinical trial (EPIK-P2, NCT04589650), is assessing efficacy, safety and pharmacokinetics of alpelisib in pediatric and adult patients with PROS. Positive preliminary results from EPIK-P1 led the FDA to grant early April 2022, an accelerated approval for alpelisib (Vijoice®) in PROS, including MCAP, for patients of 2 years of age and over, based on the efficacy (defined as a ≥20% reduction from baseline in the sum of measurable target lesion volume) observed in 37 patients from EPIK-P1 after 6 months of treatment [16]. However, these therapeutic studies preferentially targeted patients with CLOVES or Klippel-Trenaunay syndromes, and endpoints were not designed to assess neurocognitive improvement. Current data on alpelisib efficacy and safety in MCAP patients solely comes from compassionate use, which fails to demonstrate a clear benefit of the treatment on neurocognitive symptoms [15, 17]. There is even no proof that alpelisib crosses the blood-brain barrier.

To assess the clinical benefit of Alpelisb in MCAP patients, it was necessary to construct a clinical trial evaluating specifically neurocognitive functions. Based on the clinical and radiological evaluation of 33 French patients with MCAP syndrome [10], we designed the

SESAM study to assess the safety and efficacy of alpelisib in these patients, and to assess its passage across the blood-brain barrier.

METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

Study Setting

This study is promoted, partly funded, and coordinated by Dijon Bourgogne University Hospital. Drug supply and part of funding was supported by Novartis Pharmaceutical. It includes pediatric and adult patients covered by national health insurance. To minimize the logistic burden for the patients, inclusion and safety visits are performed in their local hospitals (9 sites in total). Two evaluating sites (Dijon Bourgogne University Hospital and Paris Necker Hospital) are in charge of the baseline and assessment visits.

Study design, participant, randomisation

Study design. The SESAM trial is a two-period multi-center Phase II trial, with 6 months double-blind, placebo-controlled period followed by an open label period (Figure 1). Patients and their legal representative are given by the investigator a consent form adapted to their age and ID severity (see supplemental material for the detailed consent forms) A separate and optional consent for lumbar puncture is also proposed. After validation of the screening exams at the inclusion visit, patients are randomized to take alpelisib (250mg/day for adults or 50mg/day for children) or placebo during 6 months, with a 1:1 ratio. A first evaluation will be performed at 6 months (secondary objective 1) to determine the response status and patients will be unblinded once the evaluation is done. Patients completing the double-blind phase will then be entered in an open-label phase as follows: patients on placebo will switch to alpelisib

(250mg/day for adults or 50mg/day for children), responders to alpelisib will continue alpelisib at same dose during 18 months, and non-responders will have their dose increased (300mg/day for adults, 125mg/day for children ≥6 years old only). Dose increase is not permitted for children aged 5 years and below. Non-responders are patients who did not experience sufficient clinical benefit (based on overall clinical response assessed by the investigator) and with no safety/tolerability concerns which may preclude from treatment continuation at higher dose level. Patients will undergo additional evaluation visits every 6 months, and a final evaluation visit after 24 months of alpelisib treatment (main objective). Patients will undergo a main evaluation visit with clinical, biological, functional and neurocognitive exams every 6 months. Safety monitoring will be assured on a monthly basis. The schedule and content of the visits are detailed in supplemental material (Annex 1a and 1b).

Participants. Patients aged 2-40 years, with documented evidence of postzygotic or constitutional variant(s) in *PIK3CA* and a diagnosis of MCAP with a neurocognitive disorder (from specific learning disorder to severe intellectual disability) at the time of consent could be included. The main exclusion criteria are related to contra-indications to alpelisib treatment such as history of pancreatitis, diabetes, or pneumonitis. The exhaustive list of inclusion and exclusion criteria is detailed in annex 2 of the supplemental material.

Randomisation. Randomisation will be performed by the site staff using the centralized tool in the e-CRF at baseline visit, only after confirming that the participant fulfills all the inclusion/exclusion criteria. Investigator will have to confirm the key eligibility criteria checklist embedded in the system to access to randomization tool in the e-CRF. A statistician

from Dijon Bourgogne University hospital, independent of the research, will edit the randomization list, prior to the start of the trial. Breaking of blinding can be requested by the investigator for occurrence of serious adverse events requiring knowledge of the experimental product to determine the therapeutic course to be taken, by the unblinding function in the randomization tool in the e-CRF.

Objectives

Primary objective

The primary objective is to assess the efficacy on adaptive behavior after 24 months of alpelisib treatment.

Secondary objectives

To assess: (1) the efficacy of alpelisib vs placebo on adaptive behavior based on the comparison of the proportion of participants with response at 6 months in each group, (2) the impact of alpelisib treatment on cerebral and spinal cord vascularization and volume, (3) the safety of alpelisib treatment.

Exploratory objectives

To evaluate the effects of alpelisib on: (1) the early efficacy of alpelisib on adaptive behavior , (2) quality of life and clinical global impression, (3) neuropsychological parameters, (4) epilepsy, (5) overgrowth and skin lesions when appropriate, (6) hypotonia, and (7) to quantify alpelisib passage throughout the blood-brain barrier and its relationship with systemic exposure of alpelisib.

Study Endpoints

Primary outcome

According to the publication by Chatham et al. [18], clinically meaningful improvement will be defined as a gain of at least 4 points in the Vineland II Adaptive Behavior Scale (VABS-II) at 24 months of treatment compared to baseline. The VABS-II is the most widely used scale to assess day-to-day adaptive skills, from birth to adulthood [19, 20]. It consists of a form which will be filled during an interview with an adult who is familiar with the daily living activities of the patients (usually a parent). The VABS-II is organized within a three-domain structure: communication, daily living skills, and socialization. In addition, VABS-II has a motor skills domain for children younger than 6 years of age, and an optional maladaptive behavior index [19, 20]. The domain (communication, daily living skills, and socialization) standard scores have a mean of 100 and a standard deviation of 15. Adaptive levels can also be determined. A global standard score can also be computed (the Adaptive Behavior Composite standard score) and also has a mean of 100 and a standard deviation of 15.

Secondary outcomes

For the secondary objectives, the corresponding outcomes will include:

(1) The response (yes/no) defined as an improvement of at least 4 points in the VABS-II at 6 months of treatment in the alpelisib group compared to the placebo group.

(2) The changes in brain volume, vascularization, structural connectivity, assessed by MRI, from baseline to end of treatment period.

(3) The number, type and severity of adverse events.

Exploratory outcomes

Exploratory endpoints will assess the following:

(1) Improvement of at least 4 points in the VABS-II at 6, 12, and 18 months of treatment, compared to baseline.

(2) Evolution of quality-of-life questionnaires, scores at visual analogue scale, and evolution of Clinical Global Impression of severity and Global improvement scores at 6, 12, 18, 24 months of treatment, compared to baseline.

(3) Changes in neuropsychological scales, adapted to age, at 12 and 24 months of treatment compared to baseline for attention, cognition, visuo-spatial disorders, fine motor skills, speech, reasoning and cognitive inhibition abilities, and at 24 months of treatment compared to baseline for IQ scale.

(4) Description of changes in seizures frequency (weekly diary), and antiepileptic drugs use at 6, 12, 18 and 24 months of treatment compared to baseline.

(5) Changes in overgrowth or skin lesions, classified as follows: increase, no changes or reduction in overgrowth or skin lesions according to clinical measures and evaluation of standardized photographs taken at 6, 12, 18 and 24 months of treatment compared to baseline.

(6) Changes in MFM (Motor Function Measure) scores at 6, 12, 18 and 24 months of treatment compared to baseline.

(7) Level of alpelisib (ng/mL) in cerebrospinal fluid (CSF) and in blood at between 6 or 24 months of treatment, and correlation estimate (rho) between CSF and blood levels of alpelisib.

The versions of each scale or questionnaire according to age are detailed in figure 2.

Sample size calculation:

The assumptions are as follows: 1) when following untreated MCAP patients 6.0% at best may have experienced the 4 points improvement, 2) the minimal requirement for the treatment to be considered clinically relevant by regulatory bodies is at least one third of treated patients (33%) experiencing the 4 points improvement. With alpha = 0.05, 1-beta = 0.90 and a bilateral test, 16 patients are needed to prove statistical difference between the theoretical and observed proportion. We are thus planning to enroll and analyze 20 patients to take account of possible loss of follow-up or withdrawal of consent. The approach for sample size calculation is also pragmatic, based on known and estimated cohort. A cohort of about 60 patients with MCAP and *PIK3CA* pathogenic variant is available, two thirds of them having ID or learning disability that could justify being enrolled in a clinical trial. If a patient is withdrawn from the study before treatment initiation, he will be replaced, to be able to conduct the comparison between alpelisib and placebo groups with 10 patients in each group.

PATIENT AND PUBLIC INVOLVEMENT

The feasibility of recruitment is assured by the participation of experts in the clinical and molecular aspects of PROS, including the French reference center for mosaic disorders, the RHU COSY [21] , and the French patient's association for MCAP (M-CM France).

DATA MANAGEMENT AND DATA ANALYSES

Data collection, monitoring and management

Clinical, biological and radiological data will be entered directly into a dedicated e-CRF (electronic Case Report Form) on the CleanWEB platform by the investigators, helped by Clinical Research Associate (CRA). The patient diary is paper-based and will be reviewed by

the investigator to capture the safety events that will be entered in the e-CRF. Each patient is identified by a unique code including: the number of the recruiting center, the inclusion rank, the initials of the patient (first letter of surname and first name). Automatic queries due to missing and incoherent data after data entry can be immediately generated by the CleanWEB software. Requests for corrections may also be generated by the methodological support unit of CHU Dijon and sent to the local and/or the evaluation center. The corrections will be made directly in the e-CRF by the investigators, assisted by the CRAs. Histories of changes are systematically recorded. Additionally, a CRA will perform an on-site exhaustive data monitoring for all patients. A data management plan, specific to the study, was prepared before initiating the study in the participating centers.

Statistical Analyses

Descriptive analysis

Descriptive analysis with presentation of the baseline characteristics of the cohort, with qualitative variables expressed as number of events with their frequencies (%), with their 2-sided exact 95% confidence interval (CI). For quantitative variables, mean (±SD) or median (IQR) values will be calculated.

Primary outcome analysis

Fisher's exact test or chi square test will be used to compare the observed proportion of patients reaching the primary endpoint after 24 months of treatment to the theoretical proportion of 6%. A $p<0.05$ will be considered significant.

Secondary analyses

The same approach will be used to compare the proportion of responders at M6 between alpelisib and placebo groups. Comparison of brain volumes (affected and unaffected zones) at after 24 months of treatment versus baseline will be performed using a paired-t-test or Wilcoxon rank test according to the distribution priorly assessed by a Shapiro-Wilk test.

For all safety analyses, data recorded during monitoring for adverse events (AE), either clinical or biological, will be collated and the number, type grade of AE, and their relation to treatment, will be described and the frequencies of AEs recorded as percentages and 95% CI. Results will be presented for the overall 24-month-period of treatment, and also specifically for the double-blind period, to compare safety between alpelisib and placebo. The CTCAE (v5.0) classification will be used to grade the events.

Exploratory analyses

Comparison of the mean scores of neuropsychological scale scores obtained at baseline and M24 will be performed using a paired-t-test or Wilcoxon rank test. To test the reliability of the change, the Reliable Change Index (RCI) will be calculated for each psychometric scale [22].

For fine reasoning and cognitive inhibition abilities, the raw data (logfiles from Presentation software) will be analyzed automatically using Matlab 7.1. A Reaction Time (RT) and an Error Rate (ER) analysis will be performed using R software (see supplemental material – Annex 3).

Change in scores at the MFM test will be assessed by analyzing the slopes of change between scores obtained at 6, 12, 18 and 24 months of treatment vs. baseline for each patient, and expressed as an annual rate using the unweighted least-square estimate. Comparison of mean scores will then be performed using a paired-t-test or Wilcoxon rank test.

Finally, the correlation between CSF level and blood level of alpelisib will be estimated by calculating the factor rho of Spearman's correlation.

Statistical Software

Analysis will be performed using SAS software (version 9.4). Statisticians will be blinded to the study.

METHODS: MONITORING

Harms: Steering data and safety monitoring committees

The coordinating centre at University Hospital Dijon-Bourgogne, Clinical Investigation Centre (CIC INSERM 1432), is assigned the responsibility of all study aspects: ethical, regulatory, study coordination, data management and publication strategy.

The steering committee is composed by the coordinating investigator, a methodologist, a pharmacovigilance officer, a pharmacist, a sponsor representative, and the project manager. This committee meets on a weekly basis to assess study progress and solve potential issues.

An independent data safety monitoring board (DSMB) is monitoring the patients' safety during the study and gives recommendations to the steering committee. It is composed of a pharmacologist or pharmacovigilant officer, a metholologist, a neuropediatrician, and a geneticist. The DSMB met when the first 4 included patients (25% of the initially anticipated number of patients) had completed 1 month of treatment, and will meet again at 50% and 75% of the inclusions. Treatment initiations will be halted at each threshold and will resume after review of the safety data by the DSMB.

ETHICS AND DISSEMINATION

Authorization was obtained from the French National Drug Safety Agency on 28/07/2022 and from the Ethics Committee (CPP Sud-Ouest et Outre-Mer I) on 22/09/2022 (reference number: 2022-500197-34-01). The protocol was registered with ClinicalTrials.gov under the identifier NCT05577754 in 13/10/2022. Current version of protocol is V3 (25/05/2023). The first patient was included in November 2022 and the study is expected to be completed by April 2026.

Results will be presented at scientific meetings and published in international peer-reviewed journals.

DISCUSSION

At present, there is no licensed or unlicensed drug with a proven benefit for patients with MCAP syndrome, although alpelisib FDA's approval for patients with PROS, based on collection of real-world evidence from compassionate use, makes it possible to treat patients with MCAP syndrome. As such, there is a clear unmet medical need. The understanding of the pathophysiology of the PROS and MCAP syndromes, a *PIK3CA* gain-of-function variant, and the development of specific PI3K3 inhibitors in cancer, where the same variants are found, has raised great hopes of eventually providing an effective treatment.

Whereas Novartis Pharmaceutical's sponsored EPIK-P2 is running for patients with PROS, no such study was planned for patients with MCAP syndrome. Even if the data are sparse, Venot et al. reported that the two patients with MCAP syndrome among the 19 patients with PROS syndrome, exhibited improvement in cognitive function, behavior, and cerebral perfusion [15]. We therefore hypothesized that alpelisib treatment could be useful for these patients and designed a dedicated trail.

The judgement criteria for the SESAM study were based on the collection of complete clinical and radiological data from 33 French patients, which we published in 2021 [10]. It was this cohort that led us, for example, not to adopt the evolution of epileptic seizures as the primary endpoint, as only 10% to 15% of patients suffer from them.

We chose VABS as the primary endpoint, as it has been evaluated in clinical trials on neurodevelopmental disorders, to assess the changes experienced by patients/families in their daily lives [18]. Nevertheless, in the absence of national or international consensus on the scales to be used in clinical trials aimed at demonstrating an improvement in patients with neurocognitive disorders, we have chosen to use a battery of scales, all addressing complementary domains, appropriate to the age, cognitive level, ability of the patients to concentrate, and the time constraints between two assessments. We believe that the results of this trial will provide useful information for future clinical trials targeting neurodevelopmental pathologies. We added a simplified paradigm matrix stimuli especially designed by the expert team to try to identify fine points of improvement in clinical trials that would be difficult to demonstrate with conventional scales [23]. Although we have no certainty about the possibility of achieving a reduction in brain volume, we felt it is important to assess this. Therefore, volumetric MRI will be performed at the beginning of the study and at the end of the treatment. Given the poor knowledge of the prevalence of spinal cord abnormalities in MCAP, spinal cord MRI was added to the protocol. Assessing quality of life as well as the effect of alpelisib on other non-brain manifestations of the disease were also planned.

The passage of alpelisib through the blood-brain barrier has only been hypothesized, based on the observation of a reduction in the size of brain metastases in women with breast cancer or an improvement in epileptic seizures. It is therefore necessary to assess and quantify the

passage of alpelisib into the cerebrospinal fluid (CSF), in relation with the plasmatic dosage. This will help to establish a correlation between the plasma concentration and that of the CSF, possibly allowing therapeutic monitoring on the basis of plasma concentrations alone [24].

The SESAM trial also benefits from the accumulated experience of our previous trials, one with mTOR inhibitor sirolimus, and the other with taselisib, another PI3Kα-specific inhibitor [25, 26]. In particular, conducting this trial as part of a well-established network with investigators from local centres, enabling some of the follow-up visits to be carried out close to patients' homes, improves acceptability and ensures that the trial runs smoothly.

In addition, by using this two-period design which allows all patients to be treated, we aim to demonstrate that a rigorous methodology can be applied in clinical trials in rare diseases, with high quality standards while preserving the acceptability of the trial's burden for the patient. Based on our preliminary data in CLOVES, we will enhance knowledge relating to the efficacy and side-effect profile associated with long-term treatment with *PI3K* pathway inhibition, and determine the effectiveness of treatment across an expanded number of PROS. Considering the pathological overlap deriving from the common feature of *PIK3CA* upregulation, our work may also inform future transversal therapeutic strategies in the context of a larger group of diseases.

DATASHARING

All requests for the study's data will be considered by the SESAM trial steering committee.

TRIAL STATUS

Recruitment is completed (last patient included in May2024) and follow-up is ongoing until April 2026.

REFERENCES

1. Lindhurst MJ, Parker VE, Payne F, Sapp JC, Rudge S, Harris J, et al. Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA. Nat Genet. 2012;44(8):928-33.

2. Rivière JB, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, et al. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet. 2012;44(8):934-40.

3. Kurek KC, Luks VL, Ayturk UM, Alomari AI, Fishman SJ, Spencer SA, et al. Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. Am J Hum Genet. 2012;90(6):1108-15.

4. Rios JJ, Paria N, Burns DK, Israel BA, Cornelia R, Wise CA, et al. Somatic gain-of-function mutations in PIK3CA in patients with macrodactyly. Hum Mol Genet. 2013;22(3):444-51.

5. Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. Oncogene. 2008;27(41):5486-96.

6. Kuentz P, St-Onge J, Duffourd Y, Courcet JB, Carmignac V, Jouan T, et al. Molecular diagnosis of PIK3CA-related overgrowth spectrum (PROS) in 162 patients and recommendations for genetic testing. Genet Med. 2017;19(9):989-97.

7. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, Parker VE, Blumhorst C, Darling T, et al. Clinical delineation and natural history of the PIK3CA-related overgrowth spectrum. Am J Med Genet A. 2014;164a(7):1713-33.

8. Keppler-Noreuil KM, Rios JJ, Parker VE, Semple RK, Lindhurst MJ, Sapp JC, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am J Med Genet A. 2015;167a(2):287-95.

9. Mirzaa GM, Rivière JB, Dobyns WB. Megalencephaly syndromes and activating mutations in the PI3K-AKT pathway: MPPH and MCAP. Am J Med Genet C Semin Med Genet. 2013;163c(2):122-30.

10. Garde A, Guibaud L, Goldenberg A, Petit F, Dard R, Roume J, et al. Clinical and neuroimaging findings in 33 patients with MCAP syndrome: A survey to evaluate relevant endpoints for future clinical trials. Clin Genet. 2021;99(5):650-61.

11. Haute Autorité de Santé Procotole; National de Diagnostic et de Soins for MCAP [Available from: https://www.has-sante.fr/upload/docs/application/pdf/2021-10/pnds-mcap_sept_2021.pdf.

12. Douzgou S, Rawson M, Baselga E, Danielpour M, Faivre L, Kashanian A, et al. A standard of care for individuals with PIK3CA-related disorders: An international expert consensus statement. Clin Genet. 2022;101(1):32-47.

13. Arafeh R, Samuels Y. PIK3CA in cancer: The past 30 years. Semin Cancer Biol. 2019;59:36-49.

14. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2019;380(20):1929-40.

15. Venot Q, Blanc T, Rabia SH, Berteloot L, Ladraa S, Duong JP, et al. Targeted therapy in patients with PIK3CA-related overgrowth syndrome. Nature. 2018;558(7711):540-6.

16. Food and Drug Administration - FDA approves alpelisib for PIK3CA-related overgrowth spectrum 2022 [updated 04/06/2022. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-alpelisib-pik3ca-related-overgrowth-spectrum.

17. Morin G, Degrugillier-Chopinet C, Vincent M, Fraissenon A, Aubert H, Chapelle C, et al. Treatment of two infants with PIK3CA-related overgrowth spectrum by alpelisib. J Exp Med. 2022;219(3).

18. Chatham CH, Taylor KI, Charman T, Liogier D'ardhuy X, Eule E, Fedele A, et al. Adaptive behavior in autism: Minimal clinically important differences on the Vineland-II. Autism Res. 2018;11(2):270-83.

19. Sparrow SS, Balla, D. A., & Cicchetti, D. V. . Vineland adaptive behavior scales: Interview edition, survey form manual. Circle Pines, MN: American Guidance Service. 1984.

20. Sparrow SSC, D.V.; Saulnier, C.A. Vineland-3: Vineland Adaptive Behavior Scales. 3rd ed. Pearson Assessments; Minneapolis, MN, USA. 2016.

21. Recherche Hospitalo Universitaire (RHU) - Cure Overgrowth Syndromes (COSY) [Available from: https://rhu-cosy.com/.

22. Jacobson NS, Truax P. Clinical significance: a statistical approach to defining meaningful change in psychotherapy research. J Consult Clin Psychol. 1991;59(1):12-9.

23. Curie A, Brun A, Cheylus A, Reboul A, Nazir T, Bussy G, et al. A Novel Analog Reasoning Paradigm: New Insights in Intellectually Disabled Patients. PLoS One. 2016;11(2):e0149717.

24. Batalini F, Moulder SL, Winer EP, Rugo HS, Lin NU, Wulf GM. Response of Brain Metastases From PIK3CA-Mutant Breast Cancer to Alpelisib. JCO Precis Oncol. 2020;4.

25. Parker VER, Keppler-Noreuil KM, Faivre L, Luu M, Oden NL, De Silva L, et al. Safety and efficacy of low-dose sirolimus in the PIK3CA-related overgrowth spectrum. Genet Med. 2019;21(5):1189-98.

26. Luu M, Vabres P, Devilliers H, Loffroy R, Phan A, Martin L, et al. Safety and efficacy of low-dose PI3K inhibitor taselisib in adult patients with CLOVES and Klippel-Trenaunay

syndrome (KTS): the TOTEM trial, a phase 1/2 multicenter, open-label, single-arm study. Genet Med. 2021;23(12):2433-42.

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AUTHORS' CONTRIBUTIONS :

Maxime LUU (ML) is the guarantor for the overall content. LF and PV initiated this study. LF, ML, MB designed the study. ML has written this manuscript. LF, MB, AM, AE, AG, CR, AR, RL, NH, NB, AC, LG, MC, PK, JC, GC, NBH, AC, MC and CF (sponsor), and Novartis Pharmaceuticals (sponsor) reviewed the manuscript. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

A patent application ("BYL719 (alpelisib) for use in the treatment of PIK3CA-related overgrowth spectrum" #WO2017140828A1) has been filed by INSERM (Institut National de la Santé et de la Recherche Médicale), Centre National De La Recherche Scientifique (CNRS), Université Paris Cité, and Assistance Publique-Hôpitaux De Paris (AP-HP) for the use of BYL719 (alpelisib) in the treatment of PIK3CA-related overgrowth spectrum (PROS/CLOVES syndrome). Dr. Canaud is the inventor. This patent is licensed to Novartis Pharmaceutical.

ML has received consulting fees from Novartis Pharmaceutical. GC receives or has received consulting fees from Novartis Pharmaceutical, Fresenius Medical Care, Vaderis, Alkermes, IPSEN and BridgeBio.

LEGENDS

Figure 1. Study design of the SESAM trial

Figure 2. Neuropsychological tests used in the SESAM trials. Tests were selected according to the conclusions of a working group from the DéfiScience Network (www.defiscience.fr), which assessed all the neuropsychological tests available for each domain and adapted to ID patients. The 20-item Motor Function Measure (MFM20) will be administered for children <7 yo, and the MFM32 for children ≥7yo.

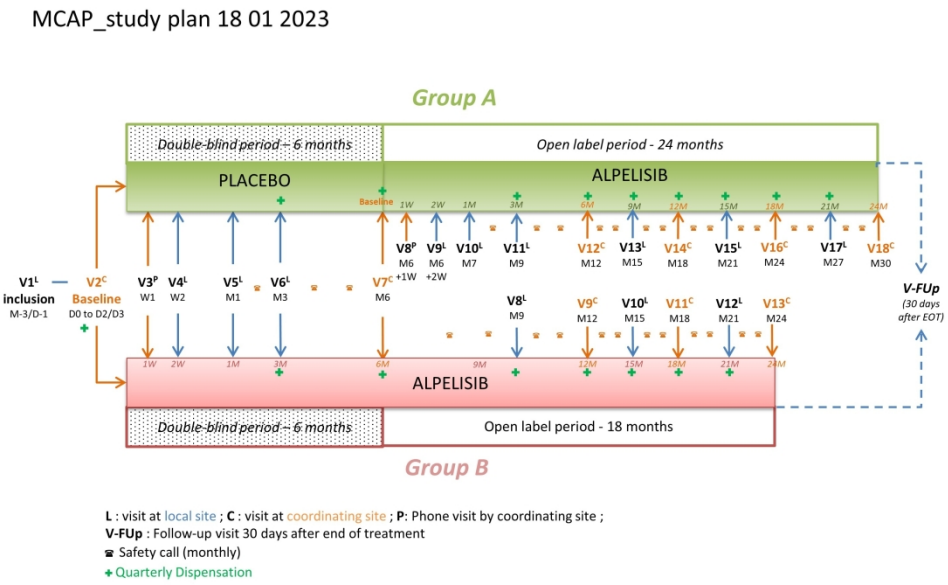


Figure 1. Study design of the SESAM trial

254x190mm (300 x 300 DPI)

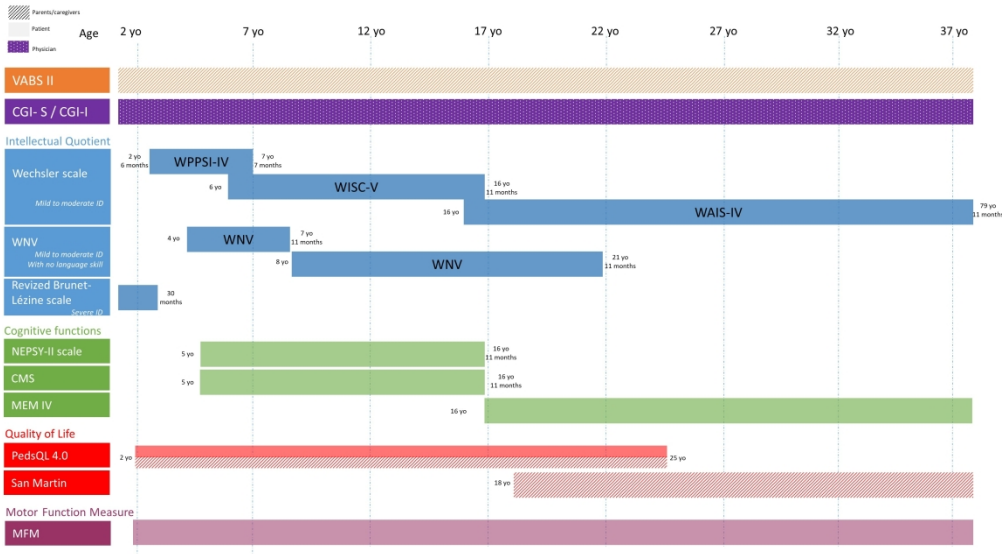


Figure 2. Neuropsychological tests used in the SESAM trials. Tests were selected according to the conclusions of a working group from the DéfiScience Network (www.defiscience.fr), which assessed all the neuropsychological tests available for each domain and adapted to ID patients. The 20-item Motor Function Measure (MFM20) will be administered for children <7 yo, and the MFM32 for children ≥7yo.

338x190mm (300 x 300 DPI)

SUPPLEMENTAL MATERIAL

Annex 1a ***Assessment Schedule for Group A : patients allocated to placebo during double-blind period***

Annex 1b. Assessment Schedule for Group B : patients allocated to alpelisib during double-blind period

Annex 2. Complete list of inclusion and exclusion criteria

Annex 3. Computer task for visual analogical reasoning paradigm

Annex 1a. Assessment Schedule for Group A : patients allocated to placebo during double-blind period

Period		Screening	Double-blind period : PLACEBO treatment						Open-label period: ALPELISIB treatment											F Up
Study duration		max 3 m	D0	W1	W2	M 1	M 3	M 6	M 6+1W	M 6+2W	M 7	M 9	M 12	M 15	M 18	M 21	M 24	M 27	M 30	M 31
treatment duration									1w	2w	1m	3 m	6 m		12 m		18 m		24 m	
Visit Name	Visit 0 Information	Visit 1 Inclusion	Visit 2 Baseline	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7 Interim evaluation Baseline	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12 Interim evaluation	Visit 13	Visit 14	Visit 15	Visit 16	Visit 17	Visit 18 End of treatment (EOT)	Visit F Up follow -up
Week	<W-12	W-12 to D-1	D0 to D2 or D3	W1	W2	W4	W12	W24	W25	W27	W28	W36	W52		W72		W104			30 days after EOT
Site	Assessment	Local	Assessment	Phone call	Local	Local	Local	Assessment	Phone call	Local	Local	Local	Assessment	Local	Assessment	Local	Assessment	Local	Assessment	Local
Window s allow ed				± 1 days	± 2 days	± 7 days	± 7 days	± 7 days	± 1 days	± 2 days	± 7 days	± 7 days	± 7 days	± 14 days	± 14 days	± 14 days	± 14 days	± 14 days	± 14 days	± 3 days
Informed Consent	Information	●																		
Inclusion/exclusion criteria	●	●																		
Demography		●																		
Medical and surgical history		●																		
Non drug therapies/surgical /medical procedures		●	Continuous up to 30 days after the last dose of study treatment																	
Concomitant medications		●	Continuous up to 30 days after the last dose of study treatment																	
Physical examination		●	●		●	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Vital Signs		●	●		●	●	●	●		●	●	●	●	●	●	●	●	●	●	●
Visual Analogue Scale			●					●									●		●	
Electrocardiogram (EKG)		●	●		●	●		●		●	●				●		●		●	
Digital Photography of affected areas			●					●							●		●		●	
Measurements of hypertrophic areas			●					●											●	
Brain MRI			●																●	
Spinal cord MRI ♦			●																●	
Blood sample		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
HbA1C		●					●	●				●		●	●		●		●	●
Hepatitis and HIV		●																		
Pregnancy test*		●	●			●	●	●			●	●	●	●	●	●	●	●	●	●
One optional lumbar puncture + blood sample for alpelisib dosages between 6 to 24months of treatment	optional consent														●		●		●	
VABS-II			●					●							●		●		●	
Wechsler scale and profil** /***			●																●	
Revised Brunet-Lézine scale ***			●																●	
NEPSY-II ***			●												●				●	
CMS/MEM***			●												●				●	
CGI-S and CGI-I			CGI-S					●							●		●		●	
Fine reasoning and inhibition abilities			●					●							●		●		●	
QoLquestionnaire ***			●					●							●		●		●	
MFM scale			●					●							●		●		●	
Randomisation			●																	
Study Drug Dispensation			●				●	●				●	●	●	●	●	●	●		
Monitoring post first administration			● during 30mins					● during 30mins												
Education of guardians on clinical signs of alpelisib toxicity/overdos			●					●												
Unblinding data								● post evaluation												
Epilepsiy daily diary			daily diary completed by patient																	
Medical diary daily dosing and any AE experienced			daily diary completed by patient																	
Adverse events		●†	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Annex 1b. Assessment Schedule for Group B : patients allocated to alpelisib during double-blind period

Period		Screening	Double-blind period : ALBELISIB treatment						Open-label period: ALPELISIB treatment						
Study duration /patient		max 3 m	D0	W1	W2	M 1	M 3	M 6	M 9	M 12	M 15	M 18	M 21	M 24	M 25
treatment duration				1w	2w	1m	3 m	6 m		12 m	15m	18m	21m	24m	
Visit Name	Visit 0 Information	Visit 1 **Inclusion**	Visit 2 **Baseline**	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7 **Interim evaluation**	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13 **End of treatment (EOT)**	Visit F Up follow -up
Week	<W-12	W-12 to D-1	D0 to D2 or D3	W1	W2	W4	W12	W24		W52				W104	30 days after EOT
Site	Assessment	Local	Assessment	Phone call	Local	Local	Local	Assessment	Local	Assessment	Local	Assessment	Local	Assessment	Local
Windows allowed				± 1 days	± 2 days	± 7 days	± 7 days	± 7 days	± 7 days	± 7 days	± 14 days	± 14 days	± 14 days	± 14 days	± 3 days
Informed Consent	Information	●													
Inclusion/exclusion criteria	●	●													
Demography		●													
Medical and surgical history		●													
Non drug therapies/surgical/medical procedures		●	Continuous up to 30 days after the last dose of study treatment												
Concomitant medications		●	Continuous up to 30 days after the last dose of study treatment												
Physical examination		●	●		●	●	●	●	●	●	●	●	●	●	●
Vital Signs		●	●		●	●	●	●	●	●	●	●	●	●	●
Visual Analogue Scale			●					●		●		●		●	
Electrocardiogram (EKG)		●	●		●	●		●		●		●		●	
Digital Photography of affected areas			●					●		●		●		●	
Measurements of hypertrophic areas			●					●						●	
Brain MRI			●											●	
Spinal cord MRI ♦			●											●	
Blood sample		●	●	●	●	●	●	●	●	●	●	●	●	●	●
HbA1C		●					●	●	●	●		●		●	●
Hepatitis and HIV		●													
Pregnancy test*		●	●			●	●	●	●	●	●	●	●	●	●
One optional lumbar puncture + blood sample for alpelisib dosages between 6 to 24months of treatment	optional consent							●		●		●		●	
VABS-II			●					●		●		●		●	
Wechsler scale and profil** /***			●											●	
Revised Brunet-Lézine scale ***			●											●	
NEPSY-II ***			●							●				●	
CMS/MEM***			●							●				●	
CGI-S and CGI-I			CGI-S					●		●		●		●	
Fine reasoning and inhibition abilities			●					●		●		●		●	
QoLquestionnaire ***			●					●		●		●		●	
MFM scale			●					●		●		●		●	
Randomisation			●												
Study Drug Dispensation			●				●	●	●	●	●	●	●		
Monitoring post first administration			●during 30 min												
Education of guardians on clinical signs of lithium toxicity/overdos			●					●							
Unblinding data								●post evaluation							
Epilepsiy daily diary			daily diary completed by patient												
Medical diary daily dosing and any AE experienced			daily diary completed by patient												
Adverse events		●[†]	●	●	●	●	●	●	●	●	●	●	●	●	●

MRI : Magnetic Resonance Imagery ; VABS-II: Vineland Adaptive Behaviour Scales second edition; HbA1C: Glycated hemoglobin; HIV: human immunodeficiency virus; NEPSY-II: A Developmental NEuroPSYchological Assessment – second edition; CMS: Children Memory Scale; CGI-S: Clinical Global Impressions Scale - Severity of illness; CGI-I : Clinical Global Impressions Scale - improvement scale; MFM : Motor Function Measure

A spinal cord sequence will be offered in parallel. It would be optional if the affected individual could not stay in the MRI for a sufficient time and if anomalies detected during the 1st imaging

* Pregnancy test can be urinary or blood, except at baseline, EoT and FuP, where blood sample is mandatory

** Wechsler scale according to patient age: WPPSI-IV (2 years et 6 months to 7 years et 7 months); or WISC-V (6 years to 16 years 11 months); or WAIS-IV (16 years to 79 years 11 months); Wechsler Nonverbal Scale of Ability (WNV)

*** adapted to age and level of ID (see schema 2)

† Only if suspected to be linked with the study procedures

Annex 2. Complete list of inclusion and exclusion criteria

1. Signed informed consent and assent (when applicable) from the patient, parent, or guardian must be obtained prior to any study related screening procedures are performed.
2. Male or female patients age ≥2 years and ≤40 years at the time of informed consent
3. Patients with diagnosis of MCAP* with neurodevelopmental disorder presentation (from specific learning disorder to severe intellectual disability)
   The most recent set of diagnostic criteria for MCAP includes five core features: progressive megalencephaly (criterion 1), developmental vascular disorders (criterion 2), distal limb anomalies (criterion 3), cortical brain malformations (criterion 4), connective tissue dysplasia (criterion 5) plus supportive features. MCAP syndrome is diagnosed in the presence of criterion 1 plus either criterion 2 or criterion 3 (Mirzaa et al., 2013). The absence of criterion 2 and 3 can be accepted in constitutional variant.
4. Documented evidence of a somatic or constitutional mutation(s) in the PIK3CA gene performed in local laboratories using a Deoxyribonucleic acid (DNA) based validated test at the time of informed consent.
5. Adequate bone marrow and organ function (assessed during the screening visit):
   a. Absolute neutrophil count ≥ 1.5 × 109/L
   b. Platelets ≥ 100 × 109/L
   c. Hemoglobin ≥ 9.0 g/dL (transfusions are allowed)
   d. Calcium (corrected for serum albumin) and magnesium within normal limits or ≤Grade 1 according to NCI-CTCAE version 5.0 if judged clinically not significant by the investigator
   e. Potassium within normal limits.
   f. INR ≤1.5
   g. Creatinine Clearance ≥ 30 mL/min using Modification of Diet in Renal Disease (MDRD) (≥18 years old) or creatinine-based Bedside Schwartz (<18 years old) Glomerular filtration rate (GFR) equation
   h. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN.
   i. Total bilirubin< ULN except for patients with Gilbert's syndrome who may only be
   j. Fasting plasma glucose (FPG) ≤ 140 mg/dL (7.7 mmol/L) and Glycosylated hemoglobin (HbA1c) ≤ 6.5% (both criteria have to be met)
   k. Fasting Serum amylase ≤ 2 × ULN
   l. Fasting Serum lipase ≤ ULN
6. Able to swallow study drug according to age: tablets, or as drinkable suspension, or granules (under development)
7. For women of child-bearing potential only: negative pregnancy test at screening visit
8. Male patients with sexual partners who are pregnant, possibly pregnant or who could become pregnant should use condoms during sexual intercourse for the duration of the study and for one week following discontinuation of alpelisib.
9. For exploratory study only: signed informed optional consent for lumbar puncture

EXCLUSION CRITERIA

Participants meeting any of the following criteria are not eligible for inclusion in this study:

1. Patient previously treated with alpelisib
2. Known impairment of GI function due to concomitant disease that may significantly alter the absorption of the study drug (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection) at time of informed consent.
3. Participant with uncontrolled diabetes mellitus (Type I or II) at time of informed consent.
4. History of hypersensitivity to any drugs or metabolites of PI3K inhibitor or any of the excipients of alpelisib at time of informed consent.
5. Participant with other concurrent severe and/or uncontrolled medical conditions that would, in the treating Physician's judgment, contraindicate administration of alpelisib (e.g., active and/or uncontrolled severe infection, chronic active hepatitis, hepatic impairment Child Pugh score C, immuno-compromised, etc.) at time of informed consent.

6. Female participants of childbearing potential and male participants who do not agree at time of informed consent to abstinence or, if sexually active, unwilling to use a condom and/or a highly effective method of contraception for the duration of the study and for one week following discontinuation of alpelisib. Highly effective contraception methods is one of the following:
    a. Total abstinence: when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
    b. Female sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or bilateral tubal ligation at least six weeks before taking alpelisib. In case of oophorectomy alone, only when the reproductive status of the female has been confirmed by follow-up hormone level assessment
    c. Male sterilization at least 6 months prior to screening. The vasectomized male partner should be the sole partner for that study participant d. Use of oral, injected or implanted hormonal methods of contraception or placement of an intrauterine device or intrauterine system or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception

   If local regulations deviate from the contraception methods listed above to prevent pregnancy, local regulations apply and will be described in the ICF.
7. Treatment by any mTOR or PI3K-AKT signaling pathway inhibitor within 1 monthbefore inclusion
8. History of prior and or ongoing malignancy (within 5 years before informed consent except radically treated Carcinoma in situ of radically treated basal-cell carcinoma of skin or thyroid gland well differentiated microcarcinoma or Stage 1 Wilms' tumor of a histology other than anaplastic), or ongoing investigations or treatment for malignancy at time of informed consent.
9. Treatment with strong inducers of CYP3A4 and inhibitors of Breast Cancer Resistance Protein (BCRP) that cannot be stopped at least the week prior to the screening
10. Debulking or other major surgery performed within 3 months at time of informed consent
11. Known history of Steven Johnson's syndrome, erythema multiform or toxic epidermal necrolysis at time of informed consent.
12. For participants ≥ 6 years of age: Participants with documented pneumonitis or interstitial lung disease at the time of informed consent and with impaired lung function (e.g., FEV1 (Forced expiratory volume) or DLCO (Diffusing Capacity of the Lung for Carbon Monoxide) ≤ 70% of predicted) that is not related to PROS.
13. For participants between 2 to 5 years of age: Participants with documented or suspicious pneumonitis or interstitial lung disease based on MRI images at time of informed consent.
14. History of acute pancreatitis within 1 year before informed consent or past medical history of chronic pancreatitis at time of informed consent.
15. Clinically significant heart disease at time of informed consent, including:
    a. History of documented congestive heart failure (New York Heart Association functional classification III-IV)
    b. Clinically significant uncontrolled cardiac arrhythmias
    c. Long QT syndrome, family history of idiopathic sudden death or congenital long QTsyndrome
    d. Corrected QT (QTcF) at screening: >470 ms for ≥18 years old / >450 ms for <18 years old
    e. Creatinine clearance < 70ml/min/1.73 m²
16. Patient currently, or in the 3 months before inclusion, enrolled in another interventional trial.
17. Person not affiliated to a national health insurance scheme
18. Patient, parents or legal authorized representative incapable of expressing consent
19. Inability to attend all trial visits
20. For the optional lumbar puncture only : known intracranial hypertension, active infection at puncture site, known coagulation disorders, Platelets < 50 × 10^9/L

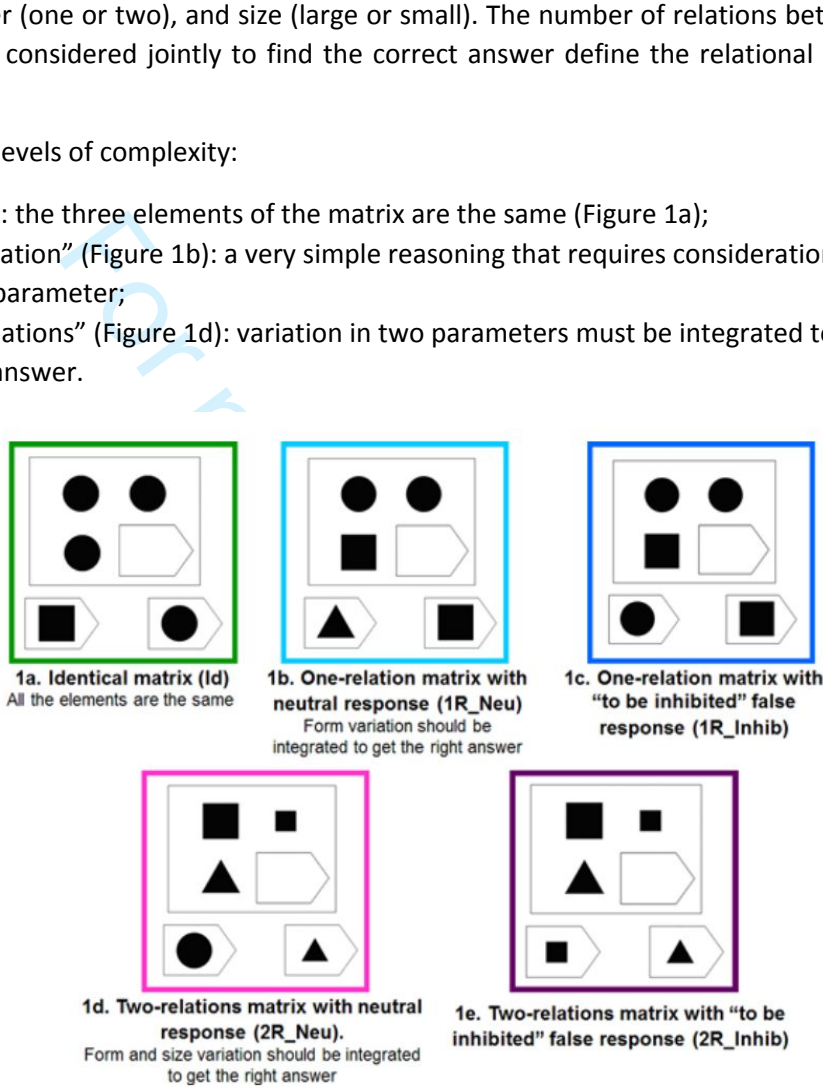
Annex 3. Computer task for visual analogical reasoning paradigm

Simple Matrices task

The simplified paradigm matrix stimuli especially designed by the expert team consist of four elements (instead of the nine cells in Raven's), with two response choices (instead of the eight in Raven's). Four parameters are involved in matrix creation: color (white, black or grey), form (round, square or triangle), number (one or two), and size (large or small). The number of relations between the items that need to be considered jointly to find the correct answer define the relational complexity of a matrix.

There are three levels of complexity:

- Identical: the three elements of the matrix are the same (Figure 1a);
- "One-relation" (Figure 1b): a very simple reasoning that requires consideration of only one varying parameter;
- "Two-relations" (Figure 1d): variation in two parameters must be integrated to get the correct answer.



Example of the 5 conditions included in the task (from Curie et al., 2016)

Furthermore, false-responses of two different types exist: a "neutral" response, which is a choice different from the items displayed in the matrix (Figure 1b and 1d) and a "to be inhibited" response, which is identical to one of the matrix items displayed (Figure 1c and 1e). Thus, this task combines 5 different conditions: identical matrices (Id), one-relation matrices with neutral responses (1R_Neu), onerelation matrices with to be inhibited responses (1R_Inhib), two-relations matrices with neutral responses (2R_Neu) and two-relations matrices with to be inhibited responses (2R_Inhib).

Task Description/data acquisition

The participants are asked to identify the missing element that completes a pattern. The stimuli are displayed on a computer screen. Participants are told to find the best answer to fill in the missing "piece". For the patients, immediate visual feedback via a happy or sad emoticon (in case of right or

wrong answer, respectively) is given during the training to make the task easier to understand. The paradigm will consist of four runs. Each matrix is preceded by a fixation cross. The subject then has to select the correct answer by pushing a button. The number of each type of condition will be counterbalanced on each run, producing the same number of right and left answers. For each type of matrix, the type of variable relation (form, color, size, number) will also be counterbalanced. The order of different conditions in the matrix display will be randomized, as is the side of the correct answer. Each run consists of 45 trials in the behavioral task and 15 trials in the eyetracking task. The matrices are generated and displayed using Presentation software (http://www.neurobs.com). A break will be allowed if necessary between two runs.