

CLINICAL REPORT

First evidence of a therapeutic effect of miransertib in a teenager with Proteus syndrome and ovarian carcinoma

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Abstract

Proteus syndrome (PS) is an ultra-rare disease characterized by progressive, disproportionate, segmental overgrowth caused by a somatic gain-of-function mutation p.Glu17Lys in the oncogene *AKT1*. The disease has high morbidity and mortality rates due to the increased risk for patients to develop cancer and progressive overgrowth. A teenage patient with severe PS phenotype developed a pelvic recurrence of low-grade serous ovarian carcinoma (LGSOC). Taking into consideration, recent results of the use of AKT inhibitors both in PS and AKT-mutant cancers, we treated the patient on a compassionate basis, with miransertib (ARQ 092), a potent, selective, allosteric AKT inhibitor. Targeted deep sequencing assay of PI3K/AKT pathway genes of the affected overgrowth lesion (cerebriform connective tissue nevus) and the tumor tissues detected the same activating *AKT1* mutation in both. Treatment with miransertib led to a complete remission of the cancer and a significant improvement in the patients' everyday life. The treatment is still ongoing at 22 months. This is the first report showing the therapeutic effects of an AKT inhibitor on both benign and malignant tissues that harbor the same pathogenic *AKT1* mutation. The present article showed that personalized medicine is feasible in ultra-rare diseases.

KEYWORDS

AKT, miransertib, ovarian cancer, personalized medicine, Proteus syndrome, target therapy

1 | INTRODUCTION

Proteus syndrome (PS) is an ultra-rare disease characterized by progressive, disproportionate, segmental overgrowth that can affect any

organ or tissue. It occurs sporadically in a mosaic form (Biesecker & Spinner, 2013; Cohen Jr., 2014). The diagnosis is based on strict clinical criteria first defined in 1999 (Biesecker, 2006; Biesecker et al., 1999) and confirmed by the presence of a somatic gain-of-function mutation c.49G>A, p.Glu17Lys in the oncogene *AKT1*, encoding the AKT1 kinase (Lindhurst et al., 2011).

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Patients with PS develop numerous severe medical complications, including vascular anomalies (capillary, venous, and lymphatic), deep venous thrombosis, pulmonary cystic malformation, severe scoliosis with megaspondyly, epilepsy, cardiac abnormalities, and renal and splenic overgrowth. Patients suffer progressive disfigurement and often die in childhood (Biesecker & Sapp, 2012).

AKT is a critical component in the PI3K/AKT/mTOR pathway, and somatic mutations in the *AKT1* gene can also act as oncogenic drivers (Carpten et al., 2007). Intriguingly, patients with PS have also a higher risk of developing both benign and malignant tumors. Although the presence of monomorphic adenomas of the parotid glands and ovarian cystadenomas (both arising before the second decade of life) have been frequently reported in patients with PS (Alves, Acosta, & Toralles, 2013; Biesecker & Sapp, 2012), these tumors have never been molecularly characterized and it is not known what role the *AKT1* (E17K) mutation may play in the development of these neoplasms.

Miransertib (ARQ 092) is an investigational orally available potent and selective allosteric pan-AKT inhibitor that inhibits both the active and inactive forms of AKT1, AKT2, and AKT3 with biochemical IC50 values of 5.0, 4.5, and 16 nM, respectively (Yu et al., 2015). Therefore, it is important to investigate it as a potential treatment for both PS and AKT-mutant cancers.

Lindhurst et al. demonstrated that miransertib reduced phosphorylation of AKT and downstream targets of AKT in a concentration-dependent manner in cells and tissues obtained from patients with PS (Lindhurst et al., 2015). Preclinical in vivo studies also showed that miransertib exhibited a strong anti-tumor activity in tumor models harboring mutant *AKT1*-E17K or with an activated AKT pathway (Yu et al., 2015).

Low doses of miransertib were shown to decrease AKT signaling in the tissue acquired from patients with PS who were enrolled in a phase I clinical study sponsored by the National Institutes of Health (NCT02594215; manuscript in press). In addition, over 150 cancer patients were also treated with miransertib in phase I trials at doses ranging from 10 to 350 mg (NCT01473095; NCT02476955). In those trials, positive responses were reported, including in patients with ovarian cancer, where either *AKT1* or different *PI3K* mutations were detected.

In this article, we report the first documented therapeutic use of an AKT1 inhibitor in a patient with PS and ovarian cancer with an *AKT1* (c.49G>A; p.Glu17Lys) mutation.

2 | CLINICAL REPORT

The patient is a 17-year-old female born to non-consanguineous parents. Pregnancy was uneventful. Progressive hand overgrowth was noticed soon after birth, prompting clinical diagnosis of Proteus syndrome. During the first 6 years of life, the patient underwent several surgical procedures for PS, including amputations of fingers in both hands, and later, she developed a progressive severe rotoscoliosis and hyperostotic fusion of all cervical vertebrae, making her wheelchair confined by the age of 10.

At the age of 13 years, the patient underwent bilateral salpingo-oophorectomy, hysterectomy, and peritoneal staging for a low-grade

serous carcinoma of the ovary (LGSOC) arising from a borderline ovarian serous tumor, with non-invasive omental implants.

Twenty months after surgical intervention, the patient developed a pelvic recurrence associated with pelvic ascites. The patient also developed a partial thrombosis of the extrahepatic segment of the portal vein, and treatment with sodium enoxaparin (4,000 IU/day) was initiated. Major surgery was deemed not feasible because of patient's multiple serious comorbidities, including vertebral synostosis in the cervical spine that significantly complicated administration of any type of anesthesia. Due to the presence of portal vein thrombosis, the Multidisciplinary Team of Fondazione Policlinico Universitario A. Gemelli did not recommend chemotherapy or hormonal therapy with an aromatase inhibitor (Grabowski et al., 2016). There were no other treatments available except non-approved, experimental therapies. The application for the Compassionate Use of the experimental drug, miransertib, was submitted to and approved by the Drugs and Therapeutics Committee of St. Vincent's Private Hospital (Dublin, Ireland). Miransertib was provided by ArQule, Inc. (Burlington MA). The treatment has been initiated when the patient was 15 years 5 months old.

2.1 | Molecular studies

2.1.1 | Targeted deep sequencing

Targeted deep sequencing assay of genes involved in the PI3K/AKT pathway genes was performed on the patient's cerebriform connective tissue nevus (CCTN) of the sole, buccal swab, blood, and tumor-derived cells. The Ion AmpliSeq Custom Panel of 21 genes involved in the PI3K/AKT/mTOR was used, as previously reported (Loconte et al., 2015). Sequencing was carried out on Ion Torrent Personal Genome Machine (ThermoFisher Scientific, TFS), using the Ion PGM Sequencing Hi-Q 200 Kit (TFS) according to the manufacturer's instructions (Loconte et al., 2015). Data analysis was performed using the Torrent Suite Software v5.0.5 (TFS). Reads were aligned to the hg19 human reference genome from the UCSC Genome Browser (<http://genome.ucsc.edu/>) and to the BED file designed using Ion AmpliSeq Designer. Alignments were visually verified with the software Alamut® v2.8.0 (Interactive Bio software). Coverage and variant analysis were conducted according to a previous report (Loconte et al., 2015).

A targeted deep sequencing assay with the Ion AmpliSeq™ Comprehensive Cancer Panel (TFS) to target all coding exons of 409 cancer-related genes in the patient's derived tumor cells and blood sample was also performed, following the manufacturer's recommended protocol.

3 | RESULTS

3.1 | AKT1 E17K mutation

The presence of the *AKT1* mutation E17K was confirmed in the patient's CCTN and in ovarian cancer. The mutant allele frequency

detected was 25.6% in the CCTN lesion and 15% in the tumor sample. No mutations have been detected in the patient's buccal swab or peripheral blood. No other mutations on genomic DNA obtained from paired tissues, cancer, blood sample, ovarian cancer-related genes included in the commercial panel (i.e., *PALB2*, *BRIP1*, *P53*, *RAD50*, *NBN*, *MRE11*, *CHEK2*, *MLH1*, *MSH2*, *PMS2*, *MSH6*) and in *BRCA1* and *BRCA2* genes, were identified. The results of the sequencing assays are described in details in Table S1 (Supplementary Data section).

3.2 | Treatment with miransertib

Following signing of informed consent by the patient and her parents, the patient underwent baseline evaluations at 15 years and 5 months of age. The full-body CT scan confirmed the presence of a secondary neoplastic mass in the pelvis (sum of the diameters: 52 mm) and multiple peritoneal implants with moderate-volume ascites (Figure 1A). CA-125 was elevated (69 U/mL). Other altered routine laboratory tests at baseline were: low platelet count ($70 \times 10^9/L$), (U.S. Department of Health and Human Services) low white cell count ($2.6 \times 10^9/L$) with neutrophil count $1.7 \times 10^9/L$; these values were probably related to PS complications such as hypersplenism and portal thrombosis. Baseline fasting glucose was within normal ranges. Baseline pretreatment assessment also included a transthoracic echocardiogram and 12-lead ECG, both were normal. Treatment with oral miransertib at 50 mg QD (daily), 5 days on/9 days off, every 2 weeks was initiated. No side effects were observed during the first 4 weeks of treatment; on week 5, based on the dose levels and schedules evaluated in oncology clinical trials, the dose of miransertib was escalated to 100 mg QD 7 days on/7 days off every 2 weeks.

Disease reassessment with full-body CT scan and CA-125 measurements were performed every 8 weeks for the first 24 weeks and every 12 weeks thereafter. CCTN lesions were photographed and visually examined at each visit, but no standardized photography assessments were implemented (Nathan et al., 2018). Adverse events were classified according to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v.5.0).

Laboratory tests, including complete blood count, blood glucose, liver, and renal function tests, were performed before the initiation of each new cycle. Self-monitoring of blood glucose using finger-stick was carried out daily from day 1 through day 8 of each cycle.

3.3 | Clinical response

Clinical improvement was observed rapidly after treatment initiation. The first disease assessment (2 months after treatment initiation) showed resolution of ascites and a significant decrease of CA-125 (38 U/mL), with stable measurable disease. CA-125 normalized at 12 weeks, and at 24 weeks, the CT showed a partial response (PR) according to RECIST 1.1 criteria, with a significant reduction in size of the pelvic mass (sum of the diameters: 39.2 mm), resolution of the peritoneal implants and complete resolution of the portal vein thrombosis (Figure 1B).

At the dose level of 100 mg of miransertib, the patient developed a transient grade 1 (CTCAE v.5.0) skin rash on the trunk, and reported

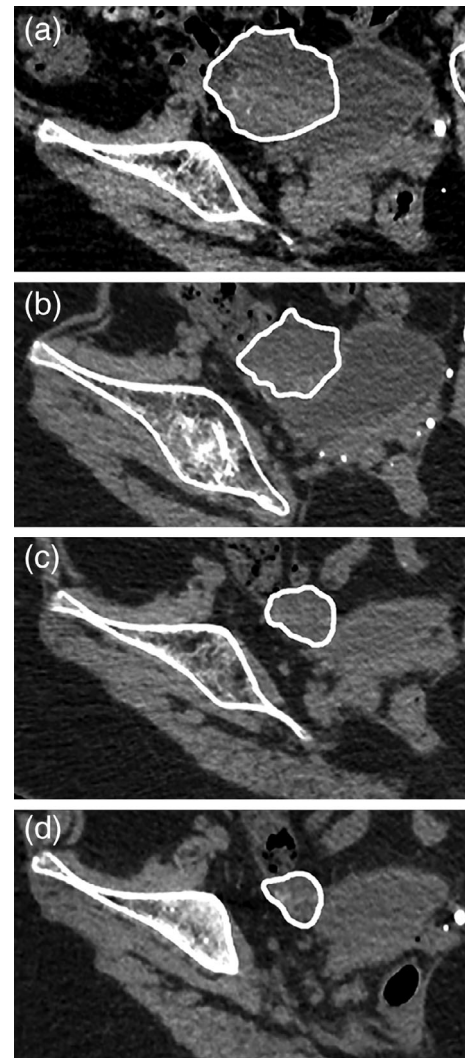


FIGURE 1 CT scans of pelvis showing decrease in xyz of LGSOC at baseline (A, RECIST 1.1: diameter 52 mm, volume 137.6 mL), at 6-month (B, RECIST 1.1: diameter 39.2 mm, volume 54.6 mL), at 12-month (C, RECIST 1.1: diameter 28.1 mm, volume 16.7 mL), at 19-month (D, RECIST 1.1: diameter 20.1 mm, volume 6.6 mL)

intermittent grade 1 nausea (especially between day 5 and 7). These were considered as unlikely related to miransertib. Grade 1–2 elevation of blood glucose and transaminases AST/GPT and ALT/GOT were also observed and considered as related to miransertib. Hyperglycemia and transaminases elevation resolved spontaneously but required occasional delay of subsequent cycles of treatment by up to 1 week. No grade 3 or grade 4 adverse events have been observed so far.

The trend in values of the selected laboratory tests (CA 125, platelet count, white blood cell count, blood glucose levels) and tumor volume are shown in Figure 2.

A CT scan at 12 and 19 month of treatment confirmed the ongoing PR with a residual pelvic mass volume measuring 16.7 mL and 6.6 mL, respectively (Figure 1C,D).

At the last follow-up performed at 22 months of treatment, the CT scan showed a complete clinical remission of the secondary lesion.

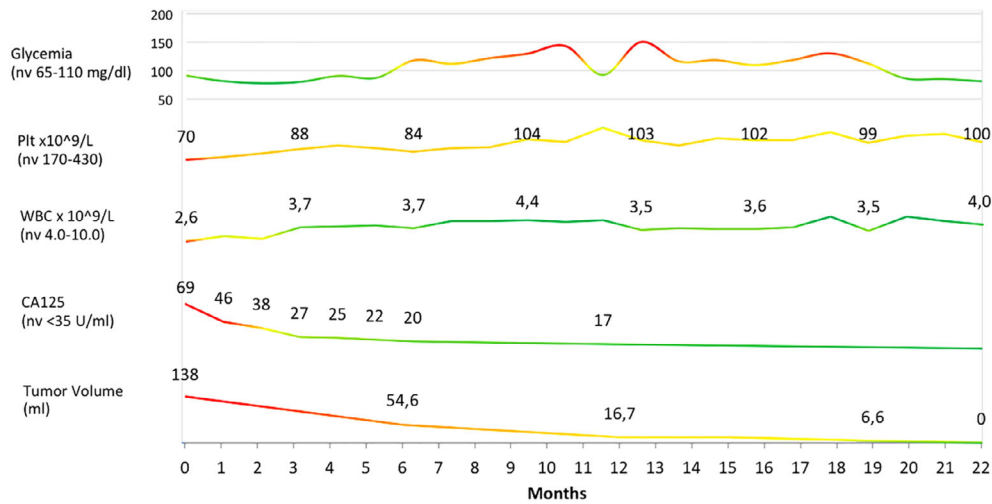


FIGURE 2 Change from baseline in CA 125, platelet count, white blood cell count, blood glucose levels, and tumor volume [Color figure can be viewed at wileyonlinelibrary.com]



FIGURE 3 Photographs at baseline and after 22 months of treatment: left hand and foot (panels A, B, C at baseline and D, E, F after 22 months of treatment), right hand and foot (panels G, H, I at baseline and L, M, N after 22 months of treatment)

CA-125 level was normal, confirming the serological remission. No formal standardized Quality of Life assessment was used; however, the patient, the parents, and the treating physicians noted improvement in her everyday life. In particular, the patient reported reduction in plantar creases of the CCNT of the left sole, increase in range of motion in forelimbs and joints (hands, spine, and knees), and this observation was confirmed by treating physicians during regular visits. Moreover, the patient reported decrease pain in spine, hands, and foot. Lastly, she was able to move herself without help from the wheelchair to the bed and to lay down in a supine position. Pictures showing the overgrowth lesions during overall treatment (hand and feet) are shown in Figure 3. The treatment with miransertib (100 mg QD, 7 days on/7 days off) is still ongoing.

4 | DISCUSSION

This article presents the first case of a successful treatment with miransertib of a patient with PS that resulted in relapsed AKT1(E17K) mutant LGSOC and clinical and serological remission after 22 months of treatment.

Although the occurrence of tumors of the female genital tract was previously reported in patients with PS, including extremely rare cases of invasive fallopian and endometrial carcinomas (Gordon, Wilroy, Lasater, & Cohen Jr., 1995), their exact incidence is extremely difficult to estimate because the molecular diagnosis of PS and its related diagnostic criteria have been defined very recently thus making it impossible to rely on historical studies and case reports.

Clinical and molecular data described in this article not only confirm the occurrence of gynecological malignancies before the second decade of life in patients with PS but also give a unique insight into the pathogenesis of these neoplasms. In fact, the diagnostic and therapeutic results reported here provide compelling evidence that in our patient, the same gene alteration of PS (AKT 1 p.Glu17Lys) is also the oncogenic driver of LGSOC. We speculate the different allele frequency found in cancer (15% in ovarian cancer tissues) versus non-cancer tissue (25.6% in CCNT lesion) could be related to the disease mechanism leading to the PS (a mosaic defect with different allele frequencies in different affected tissues). In addition, we hypothesized that the low allele frequency detected in cancer tissue, could be related to the low grade of this specific tumor (borderline serous ovarian carcinoma), and could be attributed to the fact that only a minor fraction of cancer lesion possessed the AKT mutation.

Miransertib is an experimental, orally bioavailable, and highly selective AKT-inhibitor currently tested in phase I-II trials for cancer and PS (NCT02594215, NCT03317366, NCT03317366, NCT02476955, NCT01473095). Recently, Ranieri and coauthors tested the anti-proliferative effect of ARQ 092 as compared to other PI3K/AKT/mTOR inhibitors (i.e., wortmannin, LY249002, and rapamycin) in cell lines from patients with PROS. The authors demonstrated that ARQ 092 not only had a strong anti-proliferative activity, but it was also less cytotoxic when compared to rapamycin and wortmannin (Ranieri et al., 2018). It is interesting to note that ARQ

092 was recently tested as a novel therapy for treatment of hypertrophic cardiomyopathy in Noonan syndrome with multiple lentigines due to loss of function mutation in the *PTPN11* gene. Because the SHP2Y279C/+ mice affected by significant left ventricular hypertrophy normalized the hypertrophy in 2 weeks of treatment, the authors concluded that ARQ 092 could be a promising novel therapy for the treatment of cardiac hypertrophy in individuals affected by Noonan syndrome with multiple lentigines (Wang et al., 2017).

The positive effect of miransertib described here provides evidence that targeting AKT in the context of PS, can also lead to a clinically significant and durable response in PS-associated AKT1 (E17K) mutant ovarian cancer. The treatment has been extremely well tolerated. We confirm that a clear complete remission of the tumor could have contributed to the overall improvement of our patient's everyday life. We acknowledge that the absence of quantitative imaging or photography evaluating non-neoplastic symptoms of PS (i.e., the CCTN and overgrowth) performed before therapy makes it difficult to demonstrate objectively improvement in these lesions (unfortunately, standardized serial photography, described by Nathan et al., 2018 was not available to us when the treatment of our patient was initiated). However, following multiple physical examinations and functional assessments during the treatment period, the team of treating physicians, the patient herself, and her parents reported improvement in her forelimbs, hand, and feet in term of range of motion and overgrowth lesions. Therefore, future phase 2 studies evaluating miransertib therapeutic effects with the use of quantitative endpoints (i.e., Brief Pain Inventory questionnaire for pain detection, Ped'sQL for quality of life assessment, and standardized serial photography) related to the non-neoplastic symptoms of PS are recommended.

In conclusion, personalized treatment with the AKT inhibitor miransertib in a patient with PS and a relapsed AKT1 (E17K) mutant LGSOC led to a complete clinical and serological remission of the tumor and improvement of patient's everyday life activities.

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CONFLICT OF INTEREST

GG, CL, NR, AF, RO, CC, JC, CR, GS, GZ declare no conflict of interest in relation to this manuscript. BS and GA are employee and former employee of ArQule, Inc., respectively.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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