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Pharmacodynamic Study of Miransertib in Individuals with Proteus Syndrome

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Proteus syndrome is a life-threatening segmental overgrowth syndrome caused by a mosaic gain-of-function *AKT1* variant. There are no effective treatments for Proteus syndrome. Miransertib is an AKT1 inhibitor that, prior to this study, has been evaluated only in adult oncology trials. We designed a non-randomized, phase 0/1 pilot study of miransertib in adults and children with Proteus syndrome to identify an appropriate dosage starting point for a future efficacy trial using a pharmacodynamic endpoint. The primary endpoint was a 50% reduction in the tissue levels of AKT phosphorylation from biopsies in affected individuals. We also evaluated secondary efficacy endpoints. We found that a dose of 5 mg/m^2/day (1/7 the typical dose used in oncology) led to a 50% reduction in phosphorylated AKT (pAKT) in affected tissues from five of six individuals. This dose was well tolerated. Two of the six efficacy endpoints (secondary objectives) suggested that this agent may be efficacious. We observed a decrease in a cerebriform connective tissue nevus and a reduction in pain in children. We conclude that 5 mg/m^2/day of miransertib is an appropriate starting point for future efficacy trials and that this agent shows promise of therapeutic efficacy in children with Proteus syndrome.

Introduction

Proteus syndrome (MIM: 176920) is a mosaic disorder that manifests as severe, unrelenting, progressive overgrowth[1] with major morbidity and premature mortality.[2] Proteus syndrome is caused by a somatic c.49G>A (p.Glu17Lys) activating variant in *AKT1*[3] (MIM: 164730), which is an effector of a number of growth factor receptors that signal through phosphoinositide 3-kinase (PI3K) to affect cellular growth, inhibition of apoptosis, cell migration, and glucose homeostasis.[4] The p.Glu17Lys variant activates this kinase, leading to the pathology of Proteus syndrome. While some symptomatic management strategies have been developed,[5,6] there is no primary treatment.

Because the *AKT1* c.49G>A (p.Glu17Lys) variant is somatically mutated in some cancers, small molecule AKT1 inhibitors have been developed. Miransertib (ARQ 092) is an allosteric, pan AKT inhibitor with *in vitro* IC$_{50}$ values of 5.0 nM for AKT1 (higher for AKT2 and AKT3).[7] Fibroblasts with the *AKT1* c.49G>A (p.Glu17Lys) variant treated with 31–500 nM of miransertib had reduced AKT phosphorylation, with levels at the higher three doses approaching those of quiescent wild-type cells.[8] Much higher levels (10–20 times) of the drug were necessary to reduce cell viability. Several trials of miransertib have been undertaken in adults with cancer[9,10] (ClinicalTrials.gov:

NCT02476955, NCT01473095). A classic dose escalation strategy was used to determine a maximum tolerated dose in adults of 30–60 mg/day for continuous dosing.

Based on these data, we hypothesized that miransertib could be an effective treatment for Proteus syndrome. However, the therapeutic objectives for Proteus syndrome are very different than for cancer. First, it is our goal to reduce, but not eliminate, AKT1 phosphorylation but still allow signaling that would support normal growth and other processes. Second, we expect that the therapy for this disorder would be chronic and that minimal toxicity is essential. Third, the drug must be used in children, whereas the miransertib cancer trials to date have been in adults. All of these considerations are complicated by the fact that Proteus syndrome is extremely rare, with fewer than 50 affected individuals known in North America.

These factors led us to employ a pharmacodynamically based dose escalation/de-escalation trial design, in contrast to the more typical approach to determine maximum tolerated dose. We used a combination of data from our[8] and others' (B.S., unpublished data) prior work to estimate a starting dose based on mouse tissue distribution data, demonstrating that tissue levels were about 10-fold higher than plasma levels. In addition, AKT phosphorylation was inhibited about 50% when miransertib levels in the cell

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culture media were about 30 nM.[3] Given the tissue accumulation and the plasma levels observed in cancer treatment on a phase I trial (ClinicalTrials.gov: NCT014473095), we reasoned that the starting dose for the Proteus syndrome trial should be 5 mg/m^2/day, which is 1/6–1/10 the MTD in adults with cancer. This dosage is similar to a 10 mg/day fixed dose in adult cancer trials where minimal toxicity was observed (B.S., unpublished data).

The primary endpoint for this study was a 50% reduction in pre-treatment levels of AKT phosphorylation, as measured from one of two affected tissue biopsies. We termed this the pharmacodynamically optimal dose (PDOD). While we recognized that this primary endpoint was arbitrary, we reasoned that partial inhibition of AKT1 was a reasonable objective and that 50% was more reasonable than 1%, 10%, 90%, or 99%. We also recognized that in a mosaic disorder, repeat biopsies could not be expected to have exactly the same variant allele fraction and the assay has biologic variation. We hypothesized that the 50% inhibition would be a useful starting point for a future clinical trial to measure clinical efficacy even if it has no intrinsic validity as a therapeutic outcome. We also designed secondary endpoints that would allow us to pilot several approaches that could be used to measure efficacy in future trials.

Material and Methods

Study Design and Outcomes
The design of this trial was a non-blinded, phase 0/1 dose escalation/de-escalation trial with a primary pharmacodynamic endpoint (ClinicalTrials.gov: NCT02594215). The major eligibility criteria were: age $\geq$ 12 years, met clinical diagnostic criteria for Proteus syndrome,[1] a positive clinical test result for the *AKT1* c.49G>A variant, and $\geq$1 measurable lesion (for complete list of criteria, see Table S1). Individuals were recruited from our ongoing natural history trial and through the Proteus syndrome foundation web site. Potential participants were contacted via telephone or secure email. No incentives were offered for participation. The study was approved by the NHGRI IRB and all participants (or their parents, if minors) underwent informed consent. Minors also assented to the study.

Test Article and Administration
Miransertib was provided in 5 mg capsules to be taken orally. Initial dosing (first 2 weeks) was performed as inpatients and subsequent doses administered by the affected individual or their parent on an outpatient basis.

Clinical Monitoring for Toxicities
Safety assessments included clinical examinations, laboratory evaluations, echocardiogram, and ECGs (Table S2). For the pharmacodynamic endpoint, paired skin biopsies, one split for pAKT and mutation levels and the other for miransertib tissue concentrations, were obtained before starting miransertib, 15 days after starting miransertib (C1D15), and at the end of cycle 3 (C4D1). Adherence to the dosing schedule was assessed by review of a self-completed diary and by capsule counts at each NIH visit.

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Dose-limiting toxicity (DLT) was defined as any grade $\geq$ 3 toxicity possibly, probably, or definitely related to miransertib, or persistent ($\geq$7 days) grade $\geq$ 2 miransertib-related toxicity, or any miransertib-related toxicity considered intolerable by the individual. Administration of miransertib was interrupted if a DLT occurred. Patient-Reported Outcomes and most clinical assessments were unblinded.

Photographic Quantitation of Cerebriform Connective Tissue Nevi (CCTN)
Similar lighting, distance, and camera positioning were set for each photo, and maintained for the baseline, pre-, and post-treatment photos, as best as possible accommodating leg and feet contractures. A research assistant selected the trio of images (baseline, pre-, and post-treatment) that were most similar in lighting and positioning of the foot. They were presented to the scorers without information regarding whether they were pre- or post-treatment. The area of the CCTN, including regions defined as CCTN and pre-CCTN, relative to the sole of the foot, were measured as described[11] using ImageJ version 1.48 for Mac OS X. Relative areas were measured on the sole (one individual had both feet measured) and intraclass correlation coefficients were calculated. The baseline was from our natural history data[11] and changes in area in the pre-treatment period (and in the post-treatment period for PS126, who was only treated for 8 months) were normalized to 1 year. The other post-treatment periods were not normalized as they were exactly 1 year. The evaluators were blinded to the timing/order of the images.

Quality of Life Assessments
Patient-Reported Outcome (PRO) Measures
We assessed general health-related quality of life (HRQOL), pain intensity, and pain interference pre- and post-treatment. The Impact of Pediatric Illness (IPI) Scale[12,13] was used for participants <19 years. The IPI Scale assesses adaptive behavior, emotional functioning, medical/physical status, and cognitive problems. The NRS-11 was used to assess pain intensity.[14,15] The Pain Interference Index (PII) assesses pain interference with daily activities[16,17] and was administered to children. The PROMIS Physical Function measure is a reliable and valid self-report scale for children ages 8–17 years that assesses upper extremity function and mobility[18] with parallel parent report forms.[19] For adults, the PROMIS Physical Function eight-item short form was used.[20] For each of these PROMIS measures, items were formatted on a five-point Likert scale, responses summed, and total raw scores converted to T-scores.

Tissue pAKT and AKT c.49G>A variant allele fraction levels
Skin was punch biopsied (3 mm) from an affected area of each individual before treatment, C1D15, and C4D1. Biopsies were bisected, frozen on dry ice, and stored at −80°C. Protein was isolated from half of one biopsy as described.[8] Western blot analyses were performed as described[21] using antibodies pAKT(S473) and pan-AKT (products #4060 and 2920, Cell Signaling Technologies) and imaged using an infrared imaging system (Li-Cor Biosciences). Lysates were run in triplicate on the same gel which was considered one experiment. The pAKT and pan-AKT signals for each replicate lane were obtained using Image Studio Lite Version 5.2.5 (Li-Cor Biosciences). DNA was extracted from the second piece of each biopsy using standard methods. Mutation levels were determined as described.[3]

Table 1. Treatment Emergent Adverse Events in Each Individual with Attribution

Individual Designation	Adverse Events	Severity (CTCAE v4.0 Grade)
34	9	mild (grade 1)
	2	moderate (grade 2)
38	11	mild (grade 1)
	1	moderate (grade 2)
57	10	mild (grade 1)
	3	moderate (grade 2)
	1	severe (grade 3) (unrelated)
87	10	mild (grade 1)
	2	moderate (grade 2)
101	12	mild (grade 1)
	2	moderate (grade 2)
126	7	mild (grade 1)
	7	moderate (grade 2) (probable)
	1	severe (grade 3) (probable)
Total	77	77% grade 1 21% grade 2

Most common adverse events, grade 1–2 that were possibly related: gastrointestinal (mucositis, dry mouth, and nausea); nervous system (headache); and skin (maculopapular rash). Only one individual with grades 2 and 3 adverse events, probably related: elevated liver function tests suggestive of steatohepatitis and resolved off study drug; this adverse event represented the only dose limiting toxicity in the study (during cycle 8). There were no dose-limiting toxicities in cycles 1–3, or grade 3 or higher adverse events that were definitely related. CTCAE, common toxicity criteria for adverse events.

Pharmacodynamic Quantitation of pAKT Levels

The pAKT/AKT signal was determined using technical triplicates, calculated for each lane of the western blots, and the average value and standard deviation for each lysate was calculated. The percent of averaged normalized pAKT signal at either C1D15 or C4D1 compared to pre-treatment signal and the 90% confidence interval for that value was obtained using the GraphPad Software QuickCalcs calculator.

Results

Six individuals, four males and two females, ages 57, 31, 27, 17, 16, and 13 years, were enrolled in the study (clinical details in Table S3). All had the *AKT1* c.49G>A variant in one or more biopsies. Ten individuals were screened for eligibility; six were found eligible and enrolled. Two were eligible but declined participation and two were ineligible. Three adults were enrolled first for safety as miransertib had not previously been used in children. All six individuals completed dose level 1 of the study with respect to the primary pharmacodynamic endpoint of three biopsies (baseline, C1D15, and C4D1). Individual PS38 withdrew from the study at C9D15 for personal concern for risk of toxicity (although not for a recognizable clinical toxicity).

Individual PS126 had mildly elevated liver function studies on study entry and was removed from the study at C9D1 because of progressively abnormal liver function test results (ALT, grade 2, maximum value 185 U/L [normal < 30 U/L]; AST, grade 1 maximum value 87 U/L [normal < 40 U/L]; and GGT, grade 3, maximum value 251 U/L [normal value 4–24 U/L]). Although her transaminase levels did exceed 3× the upper limit of normal, her total bilirubin levels did not exceed 0.6 mg/dL (normal 0–1.2 mg/dL) and thus she did not meet Hy's law criteria for risk of drug-induced liver injury.[22] Her ALT and AST reverted to her baseline 7 weeks after miransertib discontinuation (GGT was not evaluated pre-treatment). We observed in individual PS101 what we initially reported as a grade 3 adverse event for malignancy, a breast mass, 6 months after completion of her full course (12 months) of treatment. This mass was evaluated on biopsy to be a benign intraductal papilloma similar to many other benign tumors in individuals with Proteus syndrome (L.G.B. et al., unpublished data) and the adverse event was amended to grade 2, possibly related.

Overall, toxicity of the regimen was mild to moderate with mainly grade 1 and 2 adverse effects, most of which were possibly related to the study drug (Tables 1 and S4). Treatment-emergent adverse events per individual ranged from 7–12 grade 1, 1–7 grade 2, and 0–1 grade 3. Most common were dry mouth, mucositis, pharyngitis, sinus tachycardia, headache, and pain. Of the four individuals who completed a 12-cycle treatment period, three had 100% drug exposure and one had 92.8% drug exposure. The two individuals who discontinued treatment at C9D1 and C9D15, respectively, had 100% drug exposure.

The PDOD was the primary outcome measure of the study and was defined as meeting the 50% inhibition of pAKT compared to baseline in either the C1D15 or C4D1 biopsies, as measured by quantitative western blotting using a pAKT antibody and normalizing the results for both the pre-treatment biopsy pAKT level and the amount of total (phosphorylated plus unphosphorylated) AKT. This objective was met at dose level 1 (5 mg/m^2/day) in five of six individuals. Representative data are shown in Figure 1 (additional data in Figure S1 and Tables S5 and S6). Individual PS101 showed minimal change in pAKT levels in response to miransertib. The inhibition of pAKT levels in these six participants did not apparently correlate with the level of drug measured from the tissue samples or the plasma levels in the treated individuals (Figure 1) nor with the Variant Allele Fraction (VAF) of the *AKT1* c.49G>A alteration (Table S7). Based on meeting the PDOD objective with mild toxicity, the trial was closed after the completion of the first cohort.

We evaluated a number of secondary endpoints in this study both to explore possible endpoints for a future phase 2 study and to identify preliminary data that might show evidence of efficacy of miransertib on one of these outcomes. These included photographic quantitation of the CCTN and pre-CCTN, volumetric MRI evaluation of
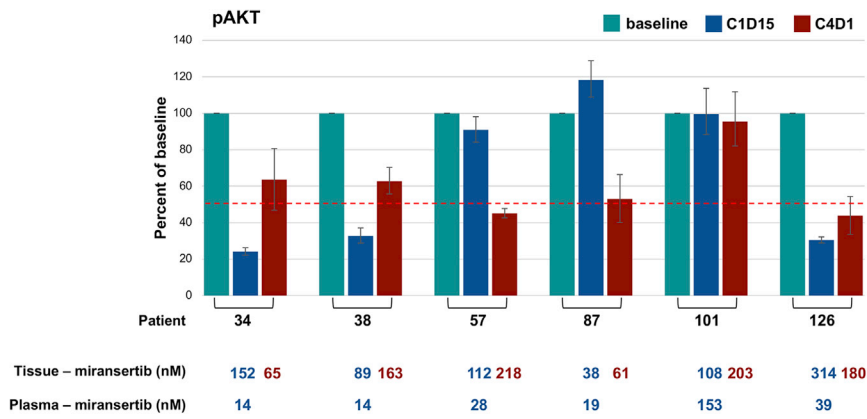
Figure 1. AKT Pharmacodynamic Inhibition with Miransertib Treatment

Levels of pAKT and miransertib in the tissue and plasma of the participants. The histogram depicts the primary outcome measure, which is the pAKT signal determined by western blot analyses in lysates obtained from biopsies of affected tissue at C1D15 (blue bars) or C4D1 (red bars) as compared to the baseline value, normalized to 100% (green bars). Lysates were tested in two separate experiments; the first dataset is shown here and both datasets are shown in Figure S1. The plasma miransertib levels obtained at C1D15 and the tissue miransertib levels at C1D15 and C4D1 are also shown numerically at the bottom of the figure. The assays were performed as technical triplicates, averaged, normalized to the baseline, and the 90% confidence intervals of the measures were compared to the 50% reduction target.

bony overgrowth and CCTN, and quality of life (QOL) assessments, including Patient-Reported Outcomes (PRO) measures and range of motion evaluations. The photographic assessment of the CCTN and pre-CCTN both by quantitative assessment and by visual qualitative inspection, showed differences between pre- and post-treatment growth rates (Figures 2A, 2B, and S2). The CCTN grew in the intervals measured before starting treatment (Figure 2, blue columns), consistent with our prior natural history study (Figure S2 and Nathan et al.[11]), which showed stability or consistent increases in CCTN over time. The qualitative observation that these lesions were stable or decreased while on drug seemed quite different from our prior natural history data. Several individuals spontaneously reported that their CCTN lesion was softer and more pliable, which they perceived to be an improvement as it was less painful when walking and was easier to clean. The volumetric MRIs were difficult to interpret (Figure S3). We encountered a number of issues with inconsistencies in imaging technique that limited the utility and precision of this assessment and the inter-observer correlation coefficients were much larger than the pre-post differences (data not shown). We are not confident that these values are precise. Each individual had from one to five bones quantitated, but there was little evidence of change in these measures and it was often difficult to precisely set the boundary of bony tissue from surrounding hypertrophic diseased tissue. The MRI assessment of the CCTN was similarly challenging (data not shown), for the same reason that boundaries were difficult to determine, and inter-observer variance was high. We cannot distinguish poor outcome measure validity from lack of efficacy for the volumetric MRI secondary outcomes.

The Patient-Reported Outcomes showed that self-reported pain declined in three individuals (Figure 3), all of whom were pediatric. Individual PS87 requested continuation of miransertib after the study because of perceived clinical benefit, including pain. The adults experienced either an increase in self-reported pain or no change (individual PS57 scored 9/10 both pre- and post-treatment, while presenting no clinical signs of pain). The mean total scores on the parent and self-report Impact of Pediatric Illness (IPI) Scales were stable for the children and adults. The intra- and inter-individual joint mobility and range of motion testing were highly variable and judged not to be useful as a future clinical endpoint (data not shown). Again, we cannot distinguish poor outcome measure validity from lack of efficacy for the range of motion secondary outcome.

Discussion

The identification of a mosaic, activating mutation in *AKT1* in Proteus syndrome provided a therapeutic target for this relentless and severe disease.[2,3] Pharmaceutical entities have developed inhibitors for this pathway because it is frequently activated in malignancies, which can be repurposed for overgrowth disorders. This repurposing presents a number of challenges such as the determination of proper dosing, therapeutic endpoints, long-term safety, etc. The disease typically manifests progression between 18 and 36 months of age through the end of adolescence.[1] We hypothesize that an effective intervention for this disease must be implemented in toddlers and maintained until adulthood, if not longer. Thus, oncology drugs primarily evaluated in short-term cancer studies in adults must undergo manifold adaptations for long-term use in children. These challenges are amplified by the rarity and phenotypic heterogeneity of Proteus syndrome, which we estimate to affect 1/1,000,000 to 1/10,000,000 individuals, with only 50–60 living individuals in the NIH natural history study.

We addressed a number of these challenges in this first pilot of a primary therapy for Proteus syndrome. We set out to determine a proper starting dose for a future efficacy trial using a pharmacodynamic assay. Combining mouse
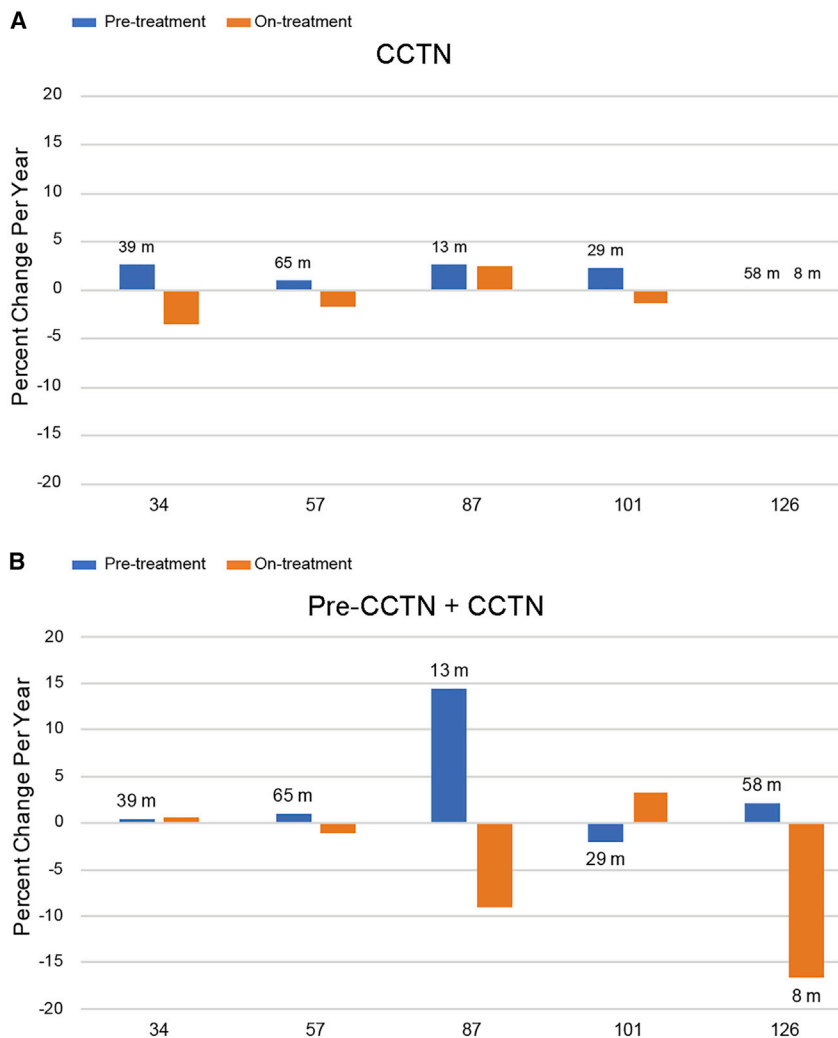
A

CCTN

Pre-treatment / On-treatment

B

Pre-CCTN + CCTN

Pre-treatment / On-treatment

Figure 2. Cerebriform Connective Tissue Nevus Changes with Miransertib Treatment

Photographic quantitation of the cerebriform connective tissue nevi (CCTN) and pre-CCTN abnormal skin comparing baseline to pre-treatment period and pre-treatment to post-treatment periods. The visible area of the entire sole, the total lesional area (pre-CCTN and CCTN combined), and CCTN area alone were quantitated using ImageJ software. (A) shows the pre-treatment and post-treatment change in the CCTN and (B) shows the pre- and post-treatment change in the combined pre-CCTN and CCTN areas.

pharmacokinetic data (B.S., unpublished data) with *in vitro* results of miransertib inhibition,[8] and adult oncology studies (ClinicalTrials.gov: NCT01473095), we estimated a starting dose for this trial of 5 mg/m^2/day, which is 1/6 the MTD in adult oncology studies. The trial was designed to assess two key variables: (1) pharmacodynamic inhibition of activated pAKT and (2) minimal AEs. Again, we note that the extreme rarity of the disorder limits the number of individuals who can be identified to enroll in such a study. We emphasize that measuring the pharmacodynamic inhibition is not necessarily a valid proxy for efficacy and that we are instead developing evidence that could inform a reasonable starting dose for an efficacy trial, not demonstrating efficacy itself.

Our initial estimation of a 5 mg/m^2/day starting dose was shown to meet the pharmacodynamic objective of 50% inhibition in the first cohort. We recognize that by choosing an outcome of 50% inhibition at either of the two endpoints (C1D15 or C4D1), we are not assessing short-term versus long-term effects. Our view is that such considerations are best evaluated in a phase II efficacy study. We also recognize that our method for quantitating the effect

of miransertib likely underestimates the biological effect. This is because the pAKT antibody is a pan-pAKT reagent that reflects the sum of AKT1, AKT2, and AKT3 phosphorylation. One of six individuals had no apparent pharmacodynamic response to miransertib. This did not appear to be related to a higher VAF nor to lower blood or tissue miransertib levels, but there was an apparent negative correlation ($R^2 = 0.83$) of relative pAKT to the ratio of tissue to blood drug levels (Figure S4). This observation is intriguing and may suggest that there are variables that affect the partitioning of drug between the vascular and tissue space, which should be addressed in a future study. We note that there is a suggestion of lower variance in pAKT ratios at the C4D1 time point as compared to the C1D15 time point (F-tests of p = 0.12 and p = 0.02 in experiments 1 and 2, respectively). One hypothesis for this is that there is a variable, but longer-term equilibration of tissue levels of the drug across individuals and that this may be related to AKT inhibition. This also warrants further study.

We observed a median of ten grade 1 (range 7–12), two grade 2 (range 1–7), and zero grade 3 (range 0–1) AE's per individual. For the four individuals who completed a 12-cycle (28 days each cycle) treatment period in the study design, no individual had less than 92% drug exposure. The two individuals who discontinued treatment at cycle 9, day 1 and cycle 9, week 32, had 100% drug exposure while on study. We cannot directly compare the rate and severity of AEs in individuals with Proteus with and without drug as no placebo arm was used. Indeed, individuals with Proteus syndrome have frequent and often serious complications of their disease—the disorder can affect any organ and tissue. The most severe AE observed in this study was grade 3 in individual PS126, an exacerbation of elevated liver function tests that were present at study enrollment, exacerbated
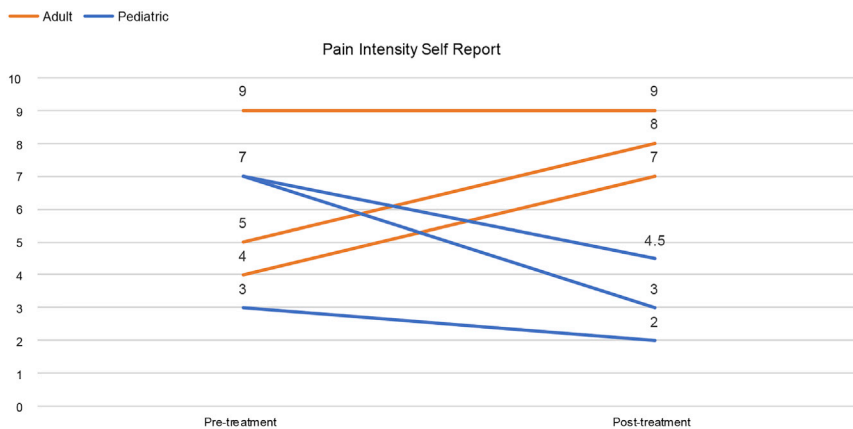
Figure 3. Pain Intensity Changes with Miransertib Treatment

Results of Pain Intensity scores comparing pre-treatment to post-treatment self-report of pain from 0 (no pain) to 10 (worst pain ever experienced). Adult participants are designated in orange; pediatric participants are designated in blue.

while on drug, and returned to baseline soon after drug withdrawal. This was assessed as probably drug related. This individual had no known alcohol consumption but was exposed to a number of other medications (Table S3). None of these medications are known to have liver dysfunction as a common or serious side effect, with the exception of acetaminophen, though she was taking this only intermittently and below the FDA-recommended maximum dose (3 g/day). A notable grade 2 AE was a benign breast mass that occurred 24 weeks after completion of the full 12-cycle study period. Both breast cancer[23] and benign tumors of the breast are recognized manifestations of Proteus syndrome. Overall, the burden of AEs was modest, especially in light of the severity of the underlying disease. While this may be expected as the dosing was much lower than in adult cancer trials, it is still valuable to assess this carefully and thoroughly, especially since several of the participants were children, an age group that has never before received this agent.

The original study design called for a dose de-escalation in the second cohort if the pAKT diminution target was met. In reviewing these data with the study team and the IRB, we elected to instead terminate the trial. Our reasoning was that the AE profile was sufficiently modest that further lowering of the dosing would be unlikely to be illuminating, even were the pharmacodynamic target to also be met at the −1 drug level. As well, the imperative to limit participant enrollment for this study and preserve potentially eligible individuals for a future efficacy study was a consideration that supported closing this trial.

Several of the secondary endpoints showed no apparent response. We piloted volumetric MRI to measure bony overgrowth and CCTN volume. Both of these endpoints showed inter-observer variation that was greater than the apparent pre-post differences. The joint mobility and range of motion evaluation was similarly variable. Our methodology was apparently inadequate to measure changes that may have occurred. It is also possible that the time period of the study was too short to observe such changes or that miransertib has no effect on bone growth and joint mobility in the age range tested here.

Several of the secondary endpoints provided useful data. These included the parent- and self-reported QOL and pain assessments, the parent- and self-reported pain assessment tools, and the photographic measurement of the CCTN. The pain assessments yielded a surprising result, which is that individual PS87 experienced a sufficient improvement in QOL and reduction of pain, which led him (and his parents) to ask to continue the drug beyond the study period because of their favorable perception of the benefit to risk ratio. Interestingly, our records do not show that this individual complained of pain before the study, but he was unambiguous that he experienced a reduction in what was apparently a chronic, tolerated level of pain and that this pain recurred at the end of the 12-month study period when he was off drug and was ameliorated again when drug was re-started after a 41-week interval off drug. It is worth noting that all three children experienced a decrease in pain (Figure 3) but two of three adults experienced an increase. This result is challenging to interpret in view of the subjectivity of pain reporting and the unblinded nature of this study. However, pain was assessed at each evaluation point without showing individuals their previous ratings or asking them whether they improved or not. There was also an apparent improvement in the CCTN and pre-CCTN lesions in several participants. It is important to note that these lesions are, in general, relentlessly progressive lesions that cause serious morbidity including intractable, severe malodor, biomechanical disruptions to shoe fitment and walking, ulceration, and pain. That we might observe arrest, much less apparent reversal of these lesions in any individual was striking. In addition, several of the participants spontaneously commented to us that their CCTN lesions were noticeably less firm, the sulci seemed less deep, and they were both more comfortable to walk on and easier to clean (re: the malodor). It is important to recognize that photographic quantitation of dermatologic lesions can be challenging. Our method may have been subject to bias in the selection of images for quantification, given the variations in position and lighting. This is somewhat mitigated by the randomization of the presentation of the images to the scorers (baseline, pre-, and post-treatment) but should be considered in future studies.

Overall, we have demonstrated a dose of miransertib in adults and children with Proteus syndrome that was well tolerated and reduced the phosphorylation of AKT in

affected individual's tissues. We also show preliminary data that it may arrest or even reverse the relentlessly progressive CCTN lesion of Proteus syndrome. Based on these encouraging data, we have terminated this study to initiate a formal efficacy study. This trial demonstrates a potentially generalizable approach to initial dose finding for non-oncologic disorders relevant to drugs targeting growth pathways that are mutated in cancer. This re-purposing of oncology drugs for non-oncologic overgrowth disorders could be important both for clinical benefit in individuals with mosaic overgrowth disorders and for improving our understanding of cancer biology.

Supplemental Data

Acknowledgments

Declaration of Interests

Web Resources

References

1. Biesecker, L. (2006). The challenges of Proteus syndrome: diagnosis and management. Eur. J. Hum. Genet. 14, 1151–1157.

2. Sapp, J.C., Hu, L., Zhao, J., Gruber, A., Schwartz, B., Ferrari, D., and Biesecker Md, L.G. (2017). Quantifying survival in patients with Proteus syndrome. Genet. Med. 19, 1376–1379.

3. Lindhurst, M.J., Sapp, J.C., Teer, J.K., Johnston, J.J., Finn, E.M., Peters, K., Turner, J., Cannons, J.L., Bick, D., Blakemore, L., et al. (2011). A mosaic activating mutation in AKT1 associated with the Proteus syndrome. N. Engl. J. Med. 365, 611–619.

4. Carpten, J.D., Faber, A.L., Horn, C., Donoho, G.P., Briggs, S.L., Robbins, C.M., Hostetter, G., Boguslawski, S., Moses, T.Y., Savage, S., et al. (2007). A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448, 439–444.

5. Biesecker, L.G. (2010). Proteus syndrome. In Management of Genetic Syndromes, S.B. Cassidy and J.E. Allanson, eds. (New York: Wiley), pp. 651–660.

6. Keppler-Noreuil, K.M., Lozier, J.N., Sapp, J.C., and Biesecker, L.G. (2017). Characterization of thrombosis in patients with Proteus syndrome. Am. J. Med. Genet. A. 173, 2359–2365.

7. Yu, Y., Savage, R.E., Eathiraj, S., Meade, J., Wick, M.J., Hall, T., Abbadessa, G., and Schwartz, B. (2015). Targeting AKT1-E17K and the PI3K/AKT pathway with an allosteric AKT inhibitor, ARQ 092. PLoS ONE 10, e0140479.

8. Lindhurst, M.J., Yourick, M.R., Yu, Y., Savage, R.E., Ferrari, D., and Biesecker, L.G. (2015). Repression of AKT signaling by ARQ 092 in cells and tissues from patients with Proteus syndrome. Sci. Rep. 5, 17162.

9. Lakhani, N., Tolcher, A.W., Patnaik, A., Rasco, D.W., O'Rourke, T.J., Schwartz, B., Abbadessa, G., Kazakin, J., Savage, R.E., Wang, Y., et al. (2017). Results of a Phase 1b Study of ARQ 092 in Combination with Carboplatin (C) Plus Paclitaxel (P) or with Paclitaxel in Patients with Solid Tumors (Chicago, IL: American Society of Clinical Oncology (ASCO) Annual Meeting).

10. Hyman, D.M., Bonafede, M., O'Cearbhaill, R., Grisham, R., Zamarin, D., Tew, W., Aghajanian, C., Cadoo, K., Friedman, C., Savage, R.E., et al. (2018). A phase 1b study of miransertib (ARQ 092) in combination with anastrozole in patients with PIK3CA or AKT1-mutant ER+ endometrial and ovarian cancer (Chicago, IL: American Association for Cancer Research (AACR) Annual Meeting).

11. Nathan, N.R., Patel, R., Crenshaw, M.M., Lindhurst, M.J., Olsen, C., Biesecker, L.G., Keppler-Noreuil, K.M., and Darling, T.N. (2018). Pathogenetic insights from quantification of the cerebriform connective tissue nevus in Proteus syndrome. J. Am. Acad. Dermatol. 78, 725–732.

12. Wolters, P., Martin, S., Walker, K., and Widemann, B. (2010). Impact of Illness (IPI) Scale parent form: Validity and reliability data for children with neurofibromatosis type 1 (NF1) and plexiform neurofibromas (PNs) (Baltimore, MD: Children's Tumor Foundation Neurofibromatosis Conference).

13. Wolters, P.S.M., Toledo-Tamula, M., Gillespie, A., Baldwin, A., and Widemann, B. (2013). Quality of life of adolescents with neurofibromatosis type 1 (NF1) and plexiform neurofibromas (PNs): Reliability and validity of the Impact of Pediatric Illness (IPI) Scale self-report form (New Orleans, LA: The National Conference in Pediatric Psychology).

14. Downie, W.W., Leatham, P.A., Rhind, V.M., Wright, V., Branco, J.A., and Anderson, J.A. (1978). Studies with pain rating scales. Ann. Rheum. Dis. 37, 378–381.

15. Miró, J., Castarlenas, E., and Huguet, A. (2009). Evidence for the use of a numerical rating scale to assess the intensity of pediatric pain. Eur. J. Pain *13*, 1089–1095.

16. Wicksell, R.K., Melin, L., Lekander, M., and Olsson, G.L. (2009). Evaluating the effectiveness of exposure and acceptance strategies to improve functioning and quality of life in longstanding pediatric pain–a randomized controlled trial. Pain *141*, 248–257.

17. Martin, S., Nelson Schmitt, S., Wolters, P.L., Abel, B., Toledo-Tamula, M.A., Baldwin, A., Wicksell, R.K., Merchant, M., and Widemann, B. (2015). Development and validation of the English Pain Interference Index and Pain Interference Index-Parent report. Pain Med. *16*, 367–373.

18. DeWitt, E.M., Stucky, B.D., Thissen, D., Irwin, D.E., Langer, M., Varni, J.W., Lai, J.S., Yeatts, K.B., and Dewalt, D.A. (2011). Construction of the eight-item patient-reported outcomes measurement information system pediatric physical function scales: built using item response theory. J. Clin. Epidemiol. *64*, 794–804.

19. Irwin, D.E., Gross, H.E., Stucky, B.D., Thissen, D., DeWitt, E.M., Lai, J.S., Amtmann, D., Khastou, L., Varni, J.W., and DeWalt, D.A. (2012). Development of six PROMIS pediatrics proxy-report item banks. Health Qual. Life Outcomes *10*, 22.

20. Rose, M., Bjorner, J.B., Gandek, B., Bruce, B., Fries, J.F., and Ware, J.E., Jr. (2014). The PROMIS Physical Function item bank was calibrated to a standardized metric and shown to improve measurement efficiency. J. Clin. Epidemiol. *67*, 516–526.

21. Lindhurst, M.J., Parker, V.E., Payne, F., Sapp, J.C., Rudge, S., Harris, J., Witkowski, A.M., Zhang, Q., Groeneveld, M.P., Scott, C.E., et al. (2012). Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in *PIK3CA*. Nat. Genet. *44*, 928–933.

22. Robles-Diaz, M., Lucena, M.I., Kaplowitz, N., Stephens, C., Medina-Cáliz, I., González-Jimenez, A., Ulzurrun, E., Gonzalez, A.F., Fernandez, M.C., Romero-Gómez, M., et al.; Spanish DILI Registry; SLatinDILI Network; and Safer and Faster Evidence-based Translation Consortium (2014). Use of Hy's law and a new composite algorithm to predict acute liver failure in patients with drug-induced liver injury. Gastroenterology *147*, 109–118.e5.

23. Iqbal, J., He, G., Biesecker, L.G., Rosen, P., Duray, P.H., Schwartzentruber, D., Beg, M., and Kahn, E. (2006). Morphological characterization of the breast in Proteus syndrome complicated by ductal carcinoma in situ. Ann. Clin. Lab. Sci. *36*, 469–474.