In vivo Efficacy of the AKT Inhibitor ARQ 092 in Noonan Syndrome With Multiple Lentigines-Associated Hypertrophic Cardiomyopathy

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ABSTRACT

Noonan Syndrome with Multiple Lentigines (NSML, formerly called LEOPARD syndrome) is an autosomal dominant "RASopathy" disorder manifesting in congenital heart disease. Most cases of NSML are caused by catalytically inactivating mutations in the protein tyrosine phosphatase (PTP), non-receptor type 11 (PTPN11) gene that encodes the SH2 domain-containing PTP-2 (SHP2). We previously generated knock-in mice harboring the PTPN11 mutation Y279C, one of the most common NSML alleles; these SHP2Y279C mice recapitulate the human disorder, and develop hypertrophic cardiomyopathy (HCM) by 12 weeks of age. Moreover, heart and/or cardiomyocyte lysates from SHP2Y279C mice exhibit increased basal and agonist-induced AKT and mTOR activities. The purpose of this present study is to assess whether the cardiac defects in SHP2Y279C mice can be reversed by treatment with ARQ 092, an oral and potent allosteric AKT inhibitor in clinical trials for patients with PI3K/AKT-driven tumors or Protein syndrome.

We obtained echocardiograms of SHP2Y279C- and wildtype (WT) littermates, either in the presence or absence of ARQ 092 at 12, 14, and 16 weeks of age. SHP2Y279C mice developed significant left ventricular hypertrophy at 12 weeks, as indicated by decreased chamber dimension and increased posterior wall thickness, which progressed further over the course of the 4 week study. Treatment of SHP2Y279C mice with ARQ 092 normalized the hypertrophy as early as 2 weeks following treatment, with hearts comparable to those in WT mice. No abnormal cardiac function was detected in hearts of WT mice, whether treated with vehicle or ARQ 092. Interestingly, we observed an increase in fractional shortening (%FS) over time in the SHP2Y279C mice, an effect of compensatory hypertrophy; this was not apparent in SHP2Y279C mice treated with ARQ 092, suggesting functional improvement upon treatment with the AKT inhibitor. SHP2Y279C mice also showed trending normalization in heart weight/body weight ratios as compared to WT, suggesting this drug may be a novel therapy for treatment of hypertension in NSML.

• SHP2, encoded by the PTPN11 gene, is a ubiquitously expressed protein-tyrosine phosphatase that contains two SH2 domains, a central PTP catalytic domain and a C-terminal tail with two tyrosine phosphorylation sites and a proline-rich motif.

• The interaction of SHP2 with pY proteins, such as receptor tyrosine kinases, cytokine receptors and scaffold adaptors, mediates downstream signaling events that control proliferation, differentiation and apoptosis.

• Germline mutations of PTPN11 cause Noonan Syndrome (NS) and Noonan Syndrome with Multiple Lentigines, formerly known as LEOPARD Syndrome. Both of these disorders have overlapping phenotypes, including congenital heart defects. However, whereas hypertrophic cardiomyopathy is rare in NS, it is the most common cardiac manifestation in NSML.

• All NS mutants tested behave as gain-of-function mutations, and act primarily to poteniate RAS/ERK pathway activation.

By contrast, NSML mutants are catalytically impaired and act as dominant negative mutants, impairing ERK activation in transient and stable transfection assays.

We hypothesize that cardiac defects in SHP2Y279C mice can be reversed by treatment with ARQ 092, an AKT inhibitor in clinical trials for patients with PI3K/AKT-driven tumors or Protein syndrome.

BACKGROUND

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RESULTS

Figure 4. Treatment with ARQ 092 reduces cardiac hypertrophy and normalizes heart size in NSML. A. Representative photographs of H&E-stained transverse heart sections from 16-week-old SHP2+/- (WT) and SHP2Y279C mice, either in the presence of vehicle or ARQ 092 AKT inhibitor (4 weeks). B. Heart weight to tissue weight ratios of 16-week-old SHP2+/- (WT) and SHP2Y279C mice, either in the presence of vehicle or ARQ 092 AKT inhibitor (4 weeks). C. Heart weights of 16-week-old SHP2+/- (WT) and SHP2Y279C mice, either in the presence of vehicle or ARQ 092 AKT inhibitor (4 weeks). D. Representative photographs of (1) H&E-stained and (b) lactic dehydrogenase-stained sections of 16-week-old SHP2+/- (WT) and SHP2Y279C mice, either in the presence of vehicle or ARQ 092 AKT inhibitor (4 weeks).

Figure 5. Treatment with the ARQ 092 drug decreases individual cardiomyocyte size in NSML hearts. Cardiac cells were measured from vehicle-stained heart sections from 16-week-old SHP2+/- (WT) and NSML mice. SHP2Y279C mice, either in the presence of vehicle or ARQ 092 AKT inhibitor (100 mg/kg/day) for 4 weeks, beginning at 12 weeks of age. *P < 0.05, where P values were derived from 2-way ANOVA with Bonferroni post hoc test when ANOVA was significant (n = 3-7 mice/group).

CONCLUSIONS

• Inhibition of AKT by ARQ 092 reduces hypertrophy in SHP2Y279C mice, as early as 2 weeks following treatment, with normalized effects observed on overall heart size, posterior wall thickness and chamber wall dimension.

• Treatment with the ARQ 092 reduces elevated fractional shortening (FS%) seen in the SHP2Y279C vehicle-treated mice, an effect often associated with pathological compensatory hypertrophy, suggesting suppression of AKT activity prevents the onset of this pathological event.

• We observe biochemical evidence for AKT inhibition in ARQ 092-treated SHP2Y279C mice, normalizing AKT/mTOR signaling in NSML mice.

• Our data suggest that inhibition of AKT activity with ARQ 092 may open a new avenue of therapeutic intervention for NSML-associated cardiac hypertrophy.

DISCLOSURES

Conflict of Interest Disclosure: The Kontaridis laboratory has received significant funds for this study from Arqule, Inc., to determine the role of ARQ 092 in NSML-associated hypertrophy.

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