**Methods**

In ARQ 092-treated (PIK3CA-related overgrowth spectrum) patients, PIK3CA germline and postzygotic mutations constitutively activate the PIK3CA/mTOR putative pathway, causing congenital segmental overgrowth, while are absent in surrounding healthy tissues (1). mTORC1 inhibitors are empirically tested in these patients, with sporadic limited benefit (2). We aim to assess the effects of pathway blockade upstream of mTOR on PIK3CA-derived tumors by utilizing the selective allosteric ARQ 092 inhibitor ARQ 092, an experimental drug with activity and long-term tolerability in cancer patients, that is also tested in patients with Proteus syndrome.

We performed targeted deep sequencing of pathway genes in six PIK3CA patient-derived cells to identify causative mutations and immunoblot to assess the phosphorylation status of AKT and its downstream targets (pS6, pPRAS40, pFOXO3, pBAD, pGSK3a-b). Anti-proliferative effect of ARQ 092 and PIK3CA/mTOR inhibitors (wortmannin, LY294002, rapamycin) was evaluated, with or without serum, in PIK3CA from six patients. ARQ 092 potently inhibited AKT signaling and exerted a strong anti-proliferative effect by inducing cell death more efficiently than comparators, with 50% of cells surviving after 60 hours of treatment at a 5 µM dose. Our data show that PIK3CA cells are ‘addicted’ to AKT and ARQ 092 treatment benefits more from AKT than mTOR inhibition. Clinical development of ARQ 092 in PIK3CA patients was warranted.

**Conclusions**

- ARQ 092 targets AKT, and its constitutive activation caused by different PIK3CA activating mutations identified in PROS patients with different phenotypes (from macroadactyly to MCPA)
- Targeting AKT permits the inhibition of the PI3K pathway immediately downstream of AKT but upstream of mTOR, achieving even better results than treatment with rapalogs (sirolimus)
- The use of an AKT inhibitor offers the possibility to circumvent additional pathways dependent on multiple and different PIK3CA classes of activating mutations
- A clinical trial with ARQ 092 in PROS patients is being planned

**Results**

**Figure 1.** Key elements of the studied pathway (PIK3, p110, PRAS40, p86) and their pharmacological inhibitors (a).

**Summary and Background**

In vitro studies with an AKT inhibitor, ARQ 092, provided evidence for a new and more effective therapeutic option in PIK3CA Related Overgrowth Spectrum (PROS) patients.

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