Synergistic Inhibition of Ovarian and Endometrial Cancer Cell Lines Using Combined Treatment of ARQ 092 and ARQ 087 in vitro and in vivo

Yi Yu, Enkeleda Nakuci, Terence Hall, Erin Chiesa, Susan Cornell-Kennon, Erica Marchlik, Brian Schwartz, Daniel T. Dransfield

ArQule, Inc. Woburn, MA, USA

RESULTS

MATERIALS AND METHODS

Synergistic Inhibition of Ovarian and Endometrial Cancer Cell Lines Using Combined Treatment of ARQ 092 and ARQ 087

ArQule, Inc. Woburn, MA, USA

CONCLUSIONS

• The combination of ARQ 087 and ARQ 092 showed synergy in 39% Ovarian and 40% Endometrial cancer cell lines tested.
• Combined treatment with ARQ 092 and ARQ 087 induced GI phase arrest in AN3CA but not IGROV-1 cells, and synergized cell proliferation through apoptosis-dependent and -independent mechanisms.
• Dysregulation of AKT and FGFR pathway was observed both in vitro and in vivo when the agents were combined.
• In vivo efficacy study of AN3CA endometrial cancer cells showed enhanced anti-tumor activity in response to ARQ 087 and ARQ 092 combination.

Ovarian and endometrial cancers are the most common female gynecological malignancies. Although traditional therapies have been utilized to reduce cancer progression, the majority of patients have developed resistant and escalating treatments. In vivo Adenocarcinoma Additive analysis treatment 0.82 R130Q, T321fs*23 and either 3 Ovary pan 0.11 1.26 0.70 were volume measurement showed changes in p both treatment G2 In vivo with treated ARQ 092 and ARQ 087 induced G1 phase arrest in AN3CA but not study, G123C width) Dysregulation AKT pathway was observed both in vitro and in vivo. Combined treatment with ARQ 092 and ARQ 087 showed enhanced anti-tumor activity. An in vitro efficacy study of AN3CA endometrial cancer cells showed enhanced anti-tumor activity in response to ARQ 087 and ARQ 092 combination.

Background: Cell lines were maintained at 37°C in a humidified atmosphere at 5% CO2, according to manufacturer's recommendations. Cell proliferation MTS assay For single agent assays, cells were seeded at an optimal density per well in 10% FBS-free media in a 96-well tissue culture plate. After 24 hours, media were replaced with fresh media containing ARQ 092 or ARQ 087 at indicated concentrations. For combination studies, cells were co-seeded with both agents at varying concentrations of ARQ 092 and ARQ 087 at a starting concentration of 100 µM. Cytometric analysis Eight ovarian and nine endometrial cancer cell lines with various genetic backgrounds were tested for ARQ 092 or ARQ 087 as single agents. The apoptosis was assessed with the annexin V-FITC and propidium iodide staining using a FACS. Determination of GI and Cytoskeleton Lab assays For the study of pharmacodynamics, the monkey microtubule instability assay in Abbe dishes. For the cytotoxicity study, the cells were seeded in 12-well tissue culture plates, treated with various concentrations of ARQ 092, ARQ 087, or ARQ 092 combination and fixed with 4% paraformaldehyde. Cells were then washed twice with PBS and stained with DAPI. Cell viability assay co-cultured with either AG538 cells or human ovarian cancer cells. Cell viability was assessed using an MTS assay. In vivo efficacy study ARQ 092 and ARQ 087 were dosed at a single dose of 100 mg/kg or 75 mg/kg daily for 12 days, co-injected with xenografts of AN3CA (human endometrial cancer) and IGROV-1 (human ovarian cancer), respectively. Cells were administered via subcutaneous injection into nude mice. Tumor burden was measured using a caliper every 3 days, and tumor volume was calculated using the following formula: Tumor volume = (Length X Width) / 2. The effects of combined treatment were analyzed by Student's t-test. The combination index (CI) was determined using the ExcelFit program. The CI values less than 0.5, 0.81, and 1.0 indicate synergistic, additive, and antagonistic effects, respectively.