Combination of AKT Inhibitor ARQ 751 with Immune Checkpoint Inhibitor and Other Therapeutic Agents

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BACKGROUND

Dysregulation of the PI3K-AKT signaling pathway is associated with many cancers. There are multiple mechanisms of AKT dysregulation in cancer including activation of receptor tyrosine kinases, gain-of-function mutations of PIK3CA, PTEN deficiency, AKT amplification and activating mutations in AKT such as AKT1-E17K. ARQ 751 is a second-generation AKT inhibitor, which has distinct physico-chemical properties compared to ArQule's first-generation inhibitor, miransertib (ARQ 092), and inhibits AKT1, 2, and 3 activity with IC_{50} values of 0.54, 0.79, and 1.3 nM, respectively. Furthermore, it binds to both active and inactive forms of AKT in an allosteric fashion and inhibits AKT activity through preventing its membrane localization and decreasing its phosphorylation in the membrane. ARQ 751 is currently in a dose-escalation phase 1 clinical study in molecularly defined cancer patients.

We assessed the combined effect of ARQ 751 with an immune checkpoint inhibitor (anti-PD-1), estrogen receptor (ER) antagonists, androgen receptor (AR) antagonist, and ARQ 531, a BTK inhibitor *in vitro* and *in vivo*.

MATERIALS AND METHODS

Reagents

ARQ 751 and ARQ 531 were synthesized at ArQule, Inc. Anastrozole, fulvestrant and enzalutamide were purchased from SelleckChem. ARQ 751 was prepared in 0.01 M phosphoric acid (pH 2.25 + 0.15) or 0.5% methyl cellulose 400 cP. Anti-PD-1 antibody was purchased from BioXcell and prepared in phosphate buffered saline.

Efficacy Study

For the combination of ARQ 751 with anti-PD-1 antibody, female BALB/c (BALB/cByJ) mice were inoculated with CT-26 mouse colon tumor cells subcutaneously and administered ARQ 751 (30 mg/kg, 5 days on, 2 days off) in combination with anti-PD-1 antibody (once every 5 days) or as a single agents for 10 days.

Cell Culture

Cancer cell lines were maintained at 37°C in a humidified atmosphere at 5% CO^2 according to manufacturer's recommendations.

Western Blot Analysis

Proteins were extracted and resolved from extracts using SDS-PAGE followed by immunoblotting. p-AKT(S473), AR and cleaved PARP were assessed. Images were captured using FuJiFilm LAS 3000.

MTS Proliferation Assay

Cells were seeded at an optimal number per well in 130 mL of full growth media in 96-well tissue culture plates, incubated overnight and subsequently treated with defined concentrations of ARQ 751 and other compounds for combination study.

Thirty microliters of the mixture of MTS reagent (18.4 mg/mL) and PMS (0.92 mg/mL) at a ratio of 20:1 was added to each well, and the plates were incubated at 37°C for 4 hours in 5% CO^2. The absorbance was measured at 490 nM using a Victor microplate reader.

Determination of Combination Index

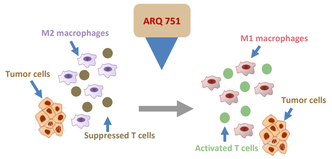
The Combination Index (CI) was determined in Activity Base using the Chou-Talalay method, with the following cut-offs applied: Strong Synergism: CI≤0.3; Synergistic: CI≤0.85; Additive: CI>0.85 to ≤1.2; and Antagonistic: CI>1.2.

RESULTS

Effect of Combined Treatment of ARQ 751 with Anti-PD-1 Antibody on Syngeneic Mouse Tumor Model

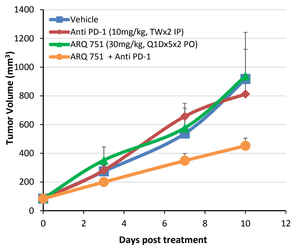
Potential enhancement of immune response through inhibition of AKT activity by ARQ 751

Inhibition of AKT by ARQ 751 may convert pro-tumor M2 macrophages to anti-tumor M1 macrophages, resulting in activation of T cell response against the tumor.



The combination of ARQ 751 with anti-PD-1 antibody showed enhanced anti-tumor activity in comparison to single agents in a CT-26 model

Syngeneic mice (BALB/cByJ) bearing CT-26 mouse colon tumor were administered ARQ 751 at 30 mg/kg 5 days on and 2 days off or anti-PD-1 antibody at 10 mg/kg twice a week as single agents or combination for 10 days.

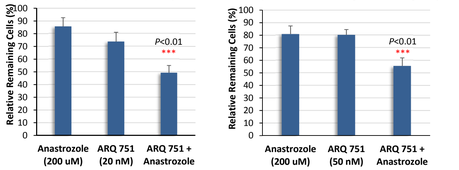


Effect of ARQ 751 Treatment Combined with ER Antagonists on Endometrial Cancer Cells

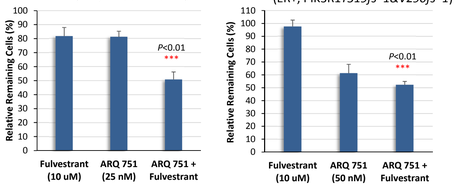
The combination of ARQ 751 with Anastrozole or Fulvestrant showed enhanced anti-proliferative activity in ER-positive endometrial cancer cells with PIK3CA/R1

The combination studies of ARQ 751 with anastrozole or fulvestrant were performed in ER-positive endometrial cancer cells with PIK3CA/R1 mutations.

Combination of ARQ 751 with Anastrozole



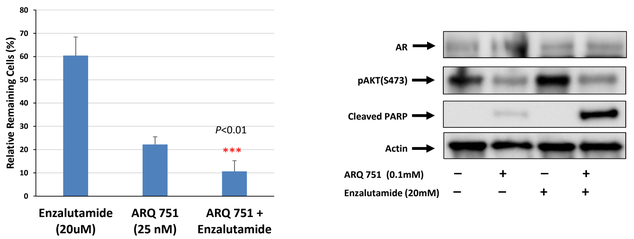
Combination of ARQ 751 with Fulvestrant



Effect of ARQ 751 Treatment Combined with Enzalutamide on Prostate Cancer Cells

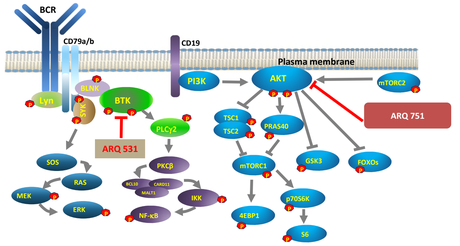
The combination of ARQ 751 with enzalutamide showed enhanced anti-proliferative activity and androgen receptor (AR) and AKT pathway inhibition in LNCaP prostate cancer cells

The combination studies of ARQ 751 with enzalutamide were performed in PTEN-deficient LNCaP prostate cancer cells. Cells were either treated with ARQ 751 in combination with enzalutamide or the single agents.



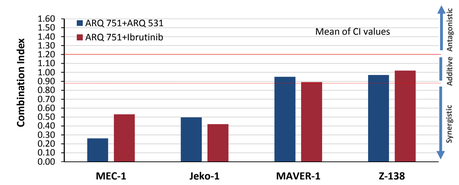
Combination of ARQ 751 with ARQ 531, a BTK Inhibitor, Is Superior to the Single Agents

Dual inhibition of AKT and BTK pathways may exert superior response to single pathway inhibition



Synergistic effect was observed in 1 CLL and 1 MCL cell lines and additive effects were observed in the other 2 MCL cell lines

The combination studies of ARQ 751 with ARQ 531 or ibrutinib were performed in 1 CLL and 3 MCL cell lines.



Combination Indices Interpretation

CI ≤	0.3	Strong Synergism
0.3 < CI ≤	0.85	Synergism
0.85 ≤ CI ≤	1.2	Additive
1.2 ≤ CI ≤	3.3	Antagonism
3.3 ≤ CI		Strong Antagonism

Mutations	CLL MEC-1	MCL Jeko-1	MCL MAVER-1	MCL Z-138
IGHV	■	■	■	■
TP53	■	■	■	
MLL2		■		
DCP1B		■		
TRPM6		■		
KIAA1671			■	

CONCLUSIONS

- Combination of ARQ 751 with anti-PD-1 antibody enhanced anti-tumor activity in a syngeneic mouse tumor model
- In ER-positive endometrial cancer cells, combination of ARQ 751 with ER antagonists (anastrozole, fulvestrant) exerts enhanced anti-proliferative effect compared to the single agents
- ARQ 751 enhances anti-proliferative activity of enzalutamide in prostate cancer cells, accompanied with inhibition of AKT pathway and increased apoptotic response
- Combination of ARQ 751 with the BTK inhibitor, ARQ 531, shows a superior anti-proliferative effect in comparison to single agents in *in vitro* anti-proliferation assays
- A phase I clinical study of ARQ 751 in a molecularly defined patient population is ongoing (NCT02761694)