In Vitro and *In Vivo* Combination of Miransertib (ARQ 092) with Anti-PD-1 Antibody, Trametinib, Lapatinib, Trastuzumab, Paclitaxel and ARQ 531

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BACKGROUND

Dysregulation of the PI3K/AKT signaling pathway has been shown to be a key driver in cancer initiation and progression. AKT is a serine/threonine kinase and a critical component in mediating the PI3K/AKT signaling axis. Although AKT inhibitors have been extensively studied, favorable clinical outcomes have been minimal. Interestingly, it has been shown that the PI3K/AKT pathway has been involved in resistance to conventional chemotherapy and targeted therapies. Thus, inhibition of AKT may reduce such resistance and improve the efficacy.

Miransertib (ARQ 092) is a potent selective allosteric pan-AKT inhibitor that is currently in clinical studies in oncology and overgrowth diseases, such as Proteus syndrome and PIK3CA-related overgrowth spectrum. We assessed the combined effect of miransertib with an immune checkpoint inhibitor (anti-PD-1), anti-MEK (trametinib) and anti-HER2 agents (lapatinib, trastuzumab), a chemotherapeutic agent (paclitaxel) and a BTK inhibitor (ARQ 531) *in vitro* and *in vivo*.

MATERIALS AND METHODS

Formulation

ARQ 092-2MSA or ARQ 092-2HCl 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 prepared in phosphate buffered saline. Trametinib was formulated in 0.5% Hydroxypropyl methylcellulose/0.2% Tween 80. Lapatinib was formulated in 0.5% Hypromellose /0.1% Tween 80. Paclitaxel was prepared in 5% glucose and trastuzumab in normal saline.

Efficacy Study

For the combination of ARQ 092 with anti-PD-1 antibody, female BALB/c (BALB/cByJ) mice were inoculated with CT-26 mouse colon tumor cells subcutaneously or implanted with 4T-1 breast tumor cells orthotopically. Tumor bearing mice were administered ARQ 092 at 60 mg/kg 5 days on and 2 days off and anti-PD-1 antibody at 10 mg/kg twice a week (CT-26) or once every 5 days (4T-1).

For the combination of ARQ 092 with HER2 antagonists, female Nude mice (BALB/cAJc1-*nu/nu*) were inoculated with KPL-4 subcutaneously and were administered ARQ 092 at 60 mg/kg for 12 days and trastuzumab at 15 mg/kg once every 6 days. Female NOD/SCID mice were inoculated with ZR-75-1 subcutaneously and were administered ARQ 092 at 20 or 40 mg/kg and lapatinib at 80 mg/kg 5 days on, 2 days off and 4 days on.

For the combination of ARQ 092 with trametinib, athymic Nude mice (Crl:NU(NCr)-Foxn1^{nu}) were inoculated with patient-derived endometrial or melanoma tumor cells. Tumor bearing mice were administered ARQ 092 at 100 mg/kg or/and trametinib at 2 mg/kg 3 days on and 4 days off for 4 weeks or ARQ 092 at 100 mg/kg 5 days on and 2 days off or/and trametinib at 3 mg/kg daily for 13 days and then 5 days on and 2 days off .

For the combination of ARQ 092 with paclitaxel, female Nude mice (BALB/cAJc1-*nu/nu*) were inoculated with KPL-4 cells or HCC1954 cells and administered ARQ 092 at 60 mg/kg for 12 days and paclitaxel every 6 days for KPL-4 model or with ARQ 092 at 120 mg/kg 5 days on and 2 days off and paclitaxel once a week for HCC1954.

The tumor length and width (mm) were measured using a digital caliper. Tumor volume (mm^3)=1/2 x (tumor length) x (tumor width)^2.

Cell Culture

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

Combination MTS 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 serial dilutions of ARQ 092 in combination with serial dilutions of ARQ 531 or ibrutinib at designated starting concentrations.

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

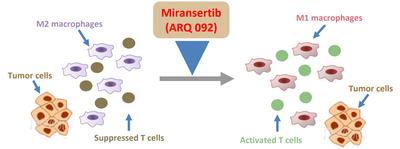
The Combination Index (CI) was determined in Activity Base using the Chou-Talalay method, with the following cut-offs being applied: Strong Synergism: CI<0.3; Synergistic: CI<0.85; Additive: CI>0.85 and <1.2; and Antagonistic: CI>1.2.

RESULTS

Effect of Combined Treatment of ARQ 092 with Anti-PD-1 Antibody on Syngeneic Mouse Tumor Model

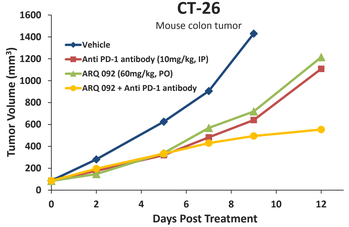
Potential enhancement of immune response through inhibition of AKT activity by ARQ 092

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



The combination of ARQ 092 with anti-PD-1 antibody showed enhanced anti-tumor activity in comparison to the single agents in the CT-26 model

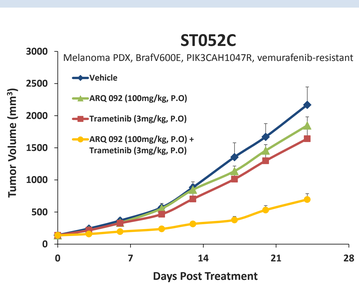
Syngeneic mice (BALB/cByJ) bearing CT-26 mouse colon tumor were administered ARQ 092 at 60 mg/kg 5 days on and 2 days off or anti-PD-1 antibody at 10 mg/kg twice a week as single agents or combination for 12 days.



Effect of Combined Treatment of ARQ 092 and Trametinib on Vemurafenib Resistant Melanoma PDX Model

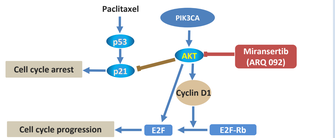
The combination of ARQ 092 and MEK inhibitor exerts enhanced antitumor activity in melanoma tumors with activated PI3K/AKT pathways and overcomes the acquired resistance to vemurafenib

Athymic Nude mice (Crl:NU(NCr)-Foxn1^{nu}) bearing patient-derived melanoma tumors were administered ARQ 092 at 100 mg/kg 5 days on and 2 days off or/and trametinib at 3 mg/kg daily for 13 days and then 5 days on and 2 days off (per week).

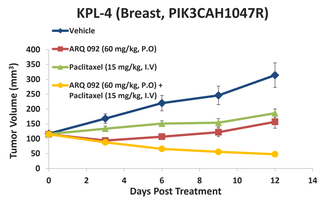


Anti-tumor Activity of Combined Treatment of ARQ 092 and Paclitaxel in Xenograft Models

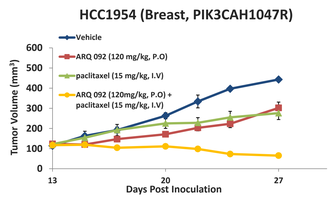
Inhibition of the AKT pathway may overcome resistance to paclitaxel



The combination of ARQ 092 with paclitaxel exerts enhanced anti-tumor activity in breast cancer cell lines with activated PI3K/AKT pathway



Female Nude mice (BALB/cAJc1-*nu/nu*) were administered ARQ 092 at 60 mg/kg for 12 days with paclitaxel every 6 days.

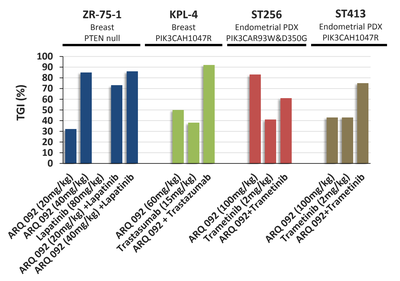


Female Nude mice (BALB/cAJc1-*nu/nu*) were administered ARQ 092 at 120 mg/kg 13 days post inoculation with 5 days on and 2 days off and paclitaxel at 15 mg/kg once a week.

Anti-tumor Activity of Combined Treatment of ARQ 092 with HER2 Antagonists or MEK Inhibitor in Xenograft models

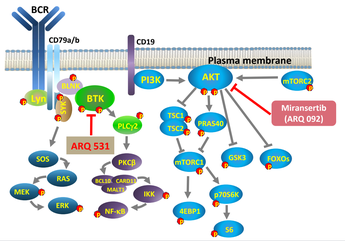
The combination of ARQ 092 with HER2 antagonists exerts enhanced anti-tumor activity in breast cancers with PIK3CA mutations/PTEN deficiency and HER2 amplification. The combination of ARQ 092 and MEK inhibitor exerts enhanced antitumor activity in endometrial cancers with an activated PI3K/AKT pathway.

Female NOD/SCID mice bearing ZR-75-1 were dosed with ARQ 092 at 20 mg/kg or 40 mg/kg and lapatinib at 80 mg/kg 5 days on 2 days off and 4 days on. Female Nude mice (BALB/cAJc1-*nu/nu*) bearing KPL-4 were dosed with ARQ 092 at 60 mg/kg for 12 days and trastuzumab at 15 mg/kg every 6 days. Athymic Nude mice (Crl:NU(NCr)-Foxn1^{nu}) bearing patient-derived endometrial tumors were dosed with ARQ 092 at 100 mg and trametinib at 2 mg/kg as single agents or in combination on a schedule of 3 days on and 4 days off for 4 weeks. TGI: tumor growth inhibition



Combination of ARQ 092 and ARQ 531, a BTK inhibitor, is Superior to Single Agents

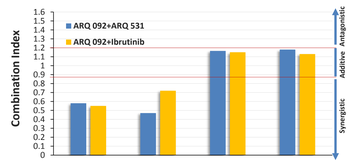
Dual inhibition of AKT and BTK pathways may exert superior response to single pathway inhibition



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 092 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 miransertib (ARQ 092) with an immune checkpoint inhibitor exhibits enhanced anti-tumor activity in syngeneic mouse tumor models
- Miransertib exhibits superior anti-tumor activity in xenograft models with cancer cells or patient-derived tumor cells when combined with paclitaxel, HER2 antagonists or MEK inhibitor
- Combination of miransertib with the BTK inhibitor, ARQ 531, shows a superior anti-proliferative effect in comparison to single agents in *in vitro* antiproliferation assays
- A phase Ib clinical study of miransertib in combination with anastrozole in a molecularly defined patient population, is ongoing (NCT02476955)