**Evaluation of fruquintinib, a potent and selective oral VEGFR inhibitor, in combination with targeted therapies or immune checkpoint inhibitors in preclinical tumor models**

Yongjun Ren, Qiaoling Sun, Jiecheng Long, Shiming Fan, Renshiang Tang, Wei Zhang, Xuetai Ge, Jianrui Tang, Lintian Wang, Dongxia Shi, Hongbo Chen, Min Cheng, Wenguang Qiong and Wegen Su

Hutchison MedPharma Ltd. Building 4, 720 Cai Lun Road, Z. Ji. Hi-Tech Park, Shanghai, China, 201203

**Abstract**

In mice, the effect of fruquintinib in combination with EGFR TKI or anti-PD-L1 is improved compared to alone treatments. The combination therapy might be contributed to the simultaneous blockade of EGFR and VEGFR in tumor cells, and this effect may lead to enhanced antitumor effect of fruquintinib in combination with anti-PD-L1.

**Materials and methods**

- **Tumor models for efficacy studies:** Patient-derived xenografts (PDX) or cell-derived xenografts (CDX) were used. Subcutaneous implanting tumor cells or tissues into Balb/c nude mice. CDX tumor cells were inoculated in syngenic Balb/c mouse.
- **Immunohistochemistry (IHC) or immunofluorescence (IP) staining in tumors:** At the end of efficacy study, tumor samples were fixed in 10% neutral buffered formalin or 4% paraformaldehyde. The 4 μm tumor sections prepared from the FFPE blocks. The staining for CD138, CD4, p-MET, CD30 and K67 were carried out, followed by biotinylated secondary antibody and the DAB chromogen. In terms of IP co-staining of PD-1, CD25, sections were manually stained with the primary antibody of CD2 and PD-1 followed by fluorophore conjugated secondary antibody.
- **Western blot for signalling inhibition in tumor tissue:** Tumor tissues (-100 mg) were homogenized in the lysates buffer. The supernatant was centrifuged at 10,000 g for 20 min at 4°C, and supernatant was collected for cell-signal detection. More than 100 μg protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a 0.4 μm polyvinylidene fluoride (PVDF) membrane. After blocking with 5% non-fat milk in TBST, PVDF membranes were incubated with anti-EGFR, pMET, p-MET, MET, ERK, ERLK, and AKT antibody followed by incubation with secondary antibodies.

**Introduction**

- The development of therapies targeting tumor angiogenesis, tumor drug resistance, and immune evasion in tumor immunotherapy has made tremendous advancement in improving overall survival (31). However, efficacy may be limited and resistance often develops, especially when targeting a single axis of tumorigenesis. Therefore, it is worthwhile to explore rational combination of therapies based on tumor-specific phenotypes.
- Fruquintinib, a potent and selective oral VEGFR inhibitor, is currently in Phase II clinical trials for non-small-cell lung cancer (NSCLC) and colorectal cancer (CRC) (NCICT2016129 and NCICT2016141H).
- It is reported here that anti-tumor effect of fruquintinib in preclinical animal tumor models in combination with therapies targeting tumor drug gene alterations such as EGFR and/or MET or with immune checkpoints inhibitor.

**Results**

- **A. Effect of fruquintinib in combination with EGFR-TKI gefitinib in NSCLC model with EGFR TKI sensitizing models**
- **B. Effect of fruquintinib in combination with a EGFR-TKI (IMPL-300) in NSCLC models with EGFR over expression or amplification**
- **C. Effect of fruquintinib in combination with MET-TKI savolitinib (JX455, AZD4566) in NSCLC or CDX, EGFR sensitizing models**

**Summary**

- In multiple xenograft models with EGFR or MET activation, fruquintinib combined with EGFR-TKI or MET-TKI significantly improved the anti-tumor activity. The enhanced anti-tumor effect in combination therapy might be attributed to the simultaneous blockade of EGFR or VEGFR in tumor cells and this effect may lead to enhanced anti-tumor effect of fruquintinib in combination with anti-PD-L1.
- In mice CT-26 syngeneic tumor model, fruquintinib treatment reduced tumor infiltration immunonoreactive cells population (CD68 positive TAMs) and decreased the CD209 on subcutaneous tumors. The growth inhibitory effect was lead to enhanced anti-tumor effect of fruquintinib in combination with anti-PD-L1.
- These results suggested that simultaneous blockade of tumor angiogenesis and tumor cell signaling or immune evasion may be a promising approach in improving treatment outcomes.

References