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

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Introduction

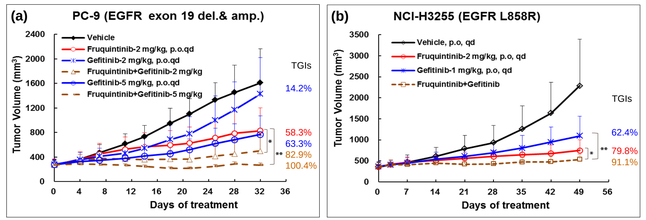
- The development of therapies targeting tumor angiogenesis, tumor driver gene alterations and tumor immune evasion has made tremendous advancement in improving overall survival [1-3] . However, efficacy may be limited and resistance often develops rapidly when targeting a single axis of tumorigenesis. 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 III clinical trials for non-small-cell lung cancer (NSCLC) and colorectal cancer (CRC) (NCT02691299 and NCT02314819)[4] .
- It is reported here that anti-tumor effect of fruquintinib in preclinical animal tumor models in combination with therapies targeting tumor driver gene alterations such as EGFR and c-MET or with immune checkpoints inhibitor.

Materials and methods

- **Tumor models for efficacy studies**: Patient-derived xenografts (PDX) or cell-derived xenografts (CDX) were used by subcutaneously implanting tumor cells or tissues into Balb/c nude mouse. CT26 tumor cells were inoculated in syngeneic Balb/c mouse.
- **Immunohistochemistry (IHC) or immunofluorescence (IF) 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 were prepared from the FFPE blocks. The staining for CD163, CD8, p-MET, CD31 and Ki67 were carried out, followed by biotinylated second antibody and the DAB chromogen. In terms of IF co-staining of PD-1 and CD8, sections were manually treated with the primary antibody of CD8 and PD-1 followed by fluorescent conjugated secondary antibody.
- **Western blot for signaling inhibition in tumor tissue**: Tumor tissues (~100 mg) were homogenized in the lysate buffer. The suspension was centrifuged and supernatants were collected for cell signaling detection. About 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 defatted milk in TBST, PVDF membrane were incubated with p-EGFR, EGFR, p-MET, MET, p-ERK, ERK, p-AKT and AKT anti-human antibodies followed by incubation with secondary antibodies.

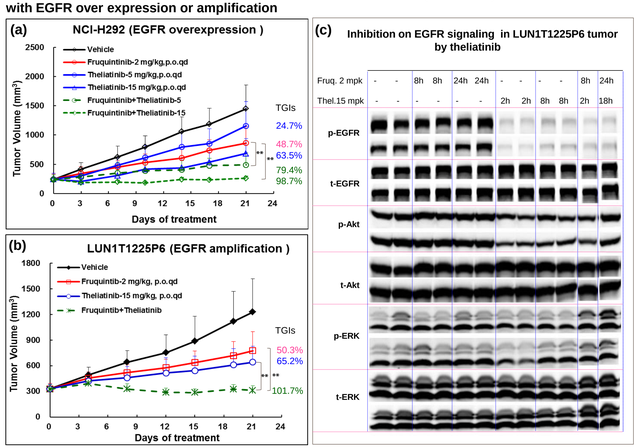
Results

A. Effect of fruquintinib in combination with EGFR-TKI gefitinib in NSCLC model with EGFR sensitizing mutation



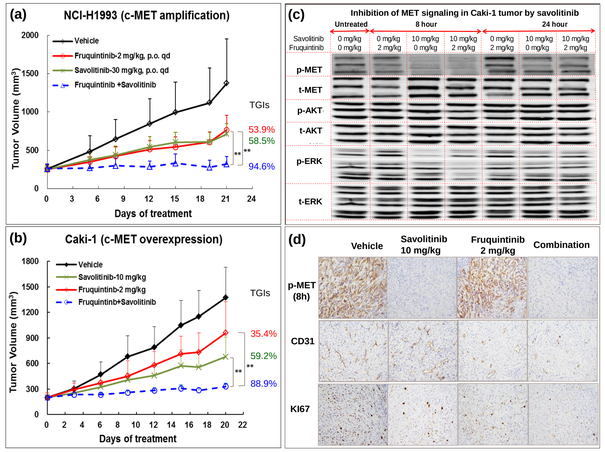
Fruquintinib and gefitinib was orally administered once a day to nude mice at the indicated dose. The enhanced anti-tumor effect was observed in combination treatment compared to either fruquintinib or gefitinib treatment alone in PC-9 **(a)** and NCI-3255 **(b)** tumor models. TGI: tumor growth inhibition; *, p<0.05; **, p<0.01.

B. Effect of fruquintinib in combination with a EGFR-TKI theliatinib (HMPL-309) in NSCLC models with EGFR over expression or amplification



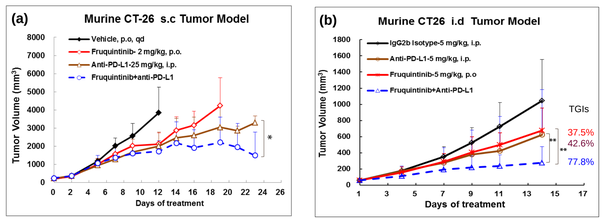
(a) NCI-H292 is a lung adenocarcinoma cell line with EGFR over expression (H score=270). **(b)** LUN1T1225 is a PDX model derived from a patient with lung squamous cell carcinoma carrying simultaneous EGFR gene amplification and high expression (H score=300). Theliatinib demonstrated potent activity against wild-type EGFR with a uniquely strong binding affinity [5] .Theliatinib is a highly selective EGFR-TKI and currently being investigated in clinical trial (NCT02601248) in China. **(c)** Monotherapy of theliatinib or combo with fruquintinib inhibited phosphorylation of EGFR and downstream signaling molecules AKT and ERK by western blot. The tumor tissue was collected at the end of the efficacy study after last dosing. TGI: tumor growth inhibition; **, p<0.01.

C. Effect of fruquintinib in combination with MET-TKI savolitinib (HMPL-504, AZD6094) in NSCLC or ccRCC xenograft models with c-MET over expression or c-MET amplification

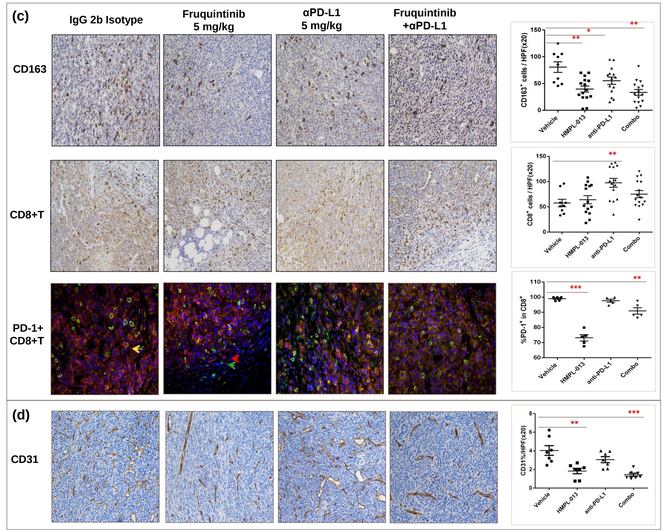


(a) NCI-H1993 is a NSCLC with MET gene amplification and high expression (H score ≥240) [6] . **(b)** Caki-1 is a ccRCC (clear cell renal cell carcinoma) model with highly expressed MET (H score=255). MET-TKI savolitinib showed moderate anti-tumor activity even at a high dose. Enhanced anti-tumor activity was achieved in both models following by fruquintinib in combination with savolitinib. TGI: tumor growth inhibition; **, p<0.01. **(c)** MET signaling analysis in Caki-1 model by Western blot following a single oral dose of savolitinib or fruquintinib and their combination. Savolitinib inhibited p-MET in a time-dependent manner[7] . **(d)** p-MET, CD31 and Ki67 in Caki-1 xenograft tumor sections were detected by IHC. Combination treatment exhibited more potent inhibition on CD31 and Ki67, compared to either of fruquintinib or savolitinib alone [7] .

D. Effect of fruquintinib in combination with anti-PD-L1 in murine CT-26 tumor models



Single agent treatment of fruquintinib (q.d.) or anti-PD-L1 (twice a week) and their combination were investigated in either CT-26 subcutaneous (s.c) **(a)** or intradermal (i.d) **(b)** syngeneic tumor models. The combination treatment resulted in improved anti-tumor efficacy. *, p<0.05; **, p<0.01.



(c) IHC or IF staining of tumor infiltrated immune cells in CT-26 i.d tumor model, the tumors were collected at the end of efficacy study. Fruquintinib decreased M2-polarized tumor-associated macrophages (CD163+) and reduced CD8+PD-1+ subsets in CD8+T population. Blue: DAPI; Red:CD8+ cells; Green:PD-1+ cells; Yellow/Orange: PD-1+CD8+ cells. **(d)** IHC staining of CD31 suggested that anti-angiogenesis effect of fruquintinib. *. p < 0.05; **, p < 0.01; ***, p < 0.001.

Summary

- In multiple xenograft models with EGFR or c-MET activation, fruquintinib combined with EGFR-TKI or MET-TKI substantially improved the anti-tumor activity. The enhanced anti-tumor effect in combination therapy might be attributed to the simultaneous blockade of EGFR or c-MET signaling in tumor cells and VEGFR suppression in the tumor microenvironment.
- In murine CT-26 syngeneic tumor model, fruquintinib treatment reduced tumor infiltrated immunosuppressive cells population (M2-polarized TAMs) and decreased the CD8+PD-1+ subsets in CD8+T population. This immuno-modulating effect may 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

1. Jayson GC et al. Expert Opin Investig Drugs. 2016;25:507-20
2. Chuang JC et al. Expert Opin Pharmacother. 2016;17:989-93
3. Reungwetwattana T et al. Lung Cancer. 2017;103:27-37
4. Cao J et al. Cancer Chemother Pharmacol. 2016;78:259-69
5. Yongxin Ren, et al. Oncotarget, 2017 [in revision]
6. Celina D'Cruz et al. Abstract 3114, AACR Annual Meeting. 2014
7. Yongxin Ren, et al. Abstract B189, AACR Annual Meeting. 2015