Synergistic effect of c-Met inhibitor Savolitinib in combination with a VEGFR inhibitor Fruquintinib in clear cell renal cell carcinoma xenograft models

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Abstract # B189

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Introduction

- Renal cell carcinoma (RCC) is the most common type of kidney tumor in human, of which approximately 80~85% is clear cell renal cell carcinoma (ccRCC)[1].
- VEGF/VEGFR targeted therapies brought significant advances in the treatment of RCC. However, resistance ultimately occurs in most cases following a transient period of clinical benefit[2]. The hepatocyte growth factor (HGF) receptor c-Met activation appeared to have emerged as one of the mechanisms of resistance to anti-VEGF/VEGFR therapies in ccRCC [3] . Therefore, targeting both c-Met and VEGFR pathways simultaneously may offer additional clinical benefit.
- In this report, a surveillance study of c-Met expression in treatment-naïve Chinese patients will be described. In addition, anti-tumor effect of c-Met inhibitor savolitinib (AZD6094, HMPL-504) in combination with a VEGFR inhibitor fruquintinib (HMPL-013) was evaluated in multiple ccRCC xenograft models.

Materials and methods

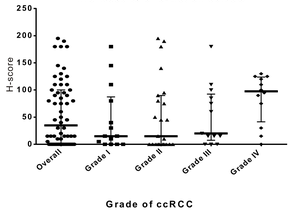
- **Human tumor samples:** ccRCC archival tumor samples from 62 treatment-naive patients were obtained from a local hospital as formalin fixed paraffin embedded (FFPE) slides.
- **PDX model development and anti-tumor efficacy study:** Fresh tumor specimens from treatment-naive patients were collected during surgery. The tumor was subcutaneously implanted into NOD-SCID mice (P0), and subsequent mouse to mouse passages were made in additional NOD-SCID or nude mice once the tumor size reached 300~500 mm^3. After several consecutive in vivo passages, the PDX models (≥P3) were used to evaluate the anti-tumor efficacy. For cell line derived xenograft models, Caki-1 and A498 cells were subcutaneously implanted into nude mice for anti-tumor efficacy evaluation.
- **Immunohistochemistry (IHC) staining of c-Met in ccRCC PDX models:** Xenograft tumor samples were fixed in 10% neutral buffered formalin, processed, embedded in paraffin and sectioned at 4 μm. Sections were manually treated with c-Met antibody (Cell Signaling Technology, #8198S), followed by biotinylated secondary antibody and the DAB chromogen.
- **Met IHC staining on ccRCC patient samples:** The IHC staining on ccRCC patient samples was performed using the CONFIRM anti-total c-Met (Ventana, SP44) rabbit mAb by Ventana Autostainer. The IHC staining was conducted in ADICON Clinical Laboratory (Shanghai).
- **Met IHC scoring system:** The whole stained section was carefully examined. The staining intensity was categorically scored on a scale of 0, 1+, 2+ or 3+. The categorical score was determined as the intensity score with the largest percentage of tumor cells. Samples scoring ⩾1+ (⩾10% tumor cells with IHC 1+ was defined as IHC+) were considered as c-Met positive. The percentage of tumor cells with positive staining was reviewed and H score was calculated. H score=100×[1×(% of 1+ cells) + 2×(% of 2+ cells) + 3×(% of 3+ cells)] [4].

Results

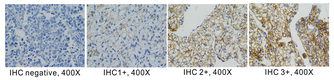
A. The summary of c-Met expression on FFPE tumor sections from 62 Chinese patients with ccRCC

Fuhrman grade	Cases (%)	MET IHC+ (%)	Median of H score	% of Hscore ⩾100
I	14 (23)	9/14 (64)	15	3/14 (21)
II	23 (37)	13/23 (57)	15	4/23 (17)
III	13 (21)	10/13 (77)	20	3/13 (23)
IV	12 (19)	11/12 (92)	97.5	6/12 (50)
Overall	62 (100)	43/62 (69)	35	16/62 (26)

⩾10% tumor cells with IHC 1+ was defined as IHC positive

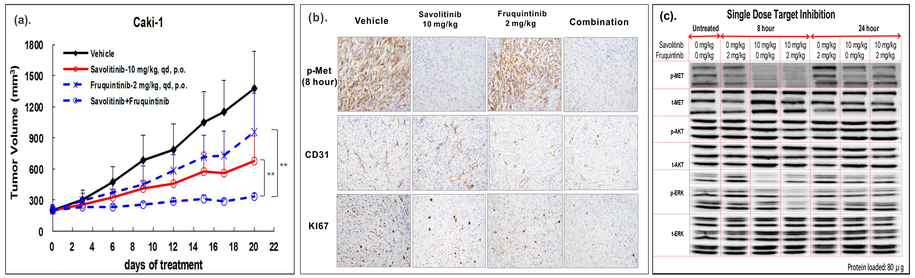


Examples of MET IHC scoring

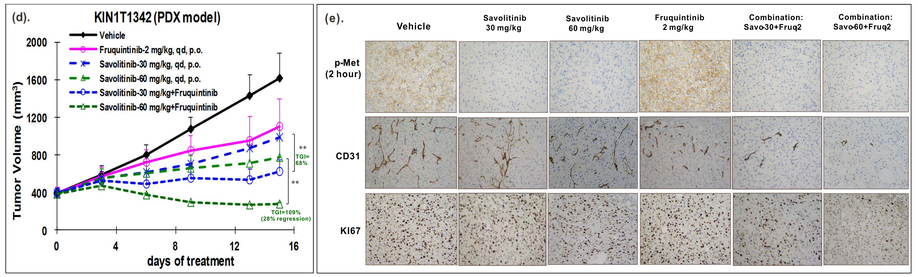


- Met expression was frequently found in ccRCC patients (69%). Among of them, 26% tumor showed H score ⩾100 .
- The median of H score in Grade IV patients was higher than that in Grade I, II, III patients but no statistical significance was shown due to the large intra-patient variation and limited sample size.

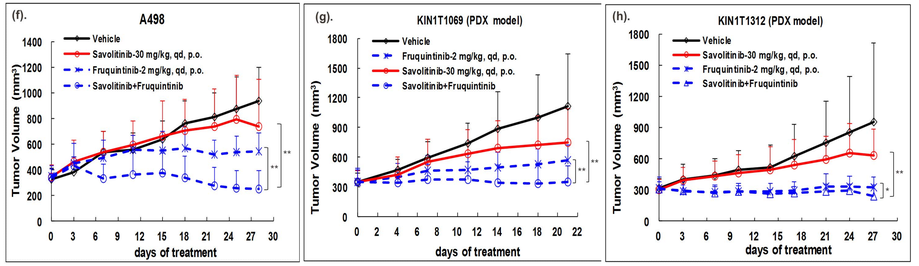
B. In vivo anti-tumor activity of savolitinib in combination with fruquintinib in ccRCC xenograft models



(a). Mice bearing established Caki-1 xenografts were treated orally with the daily indicated doses of savolitinib and fruquintinib. Tumor volume was measured three times a week. Anti-tumor efficacy measured throughout %TGI (the values are shown in the following summary table, ** represents P<0.01). **(b)**. Met phosphorylation (p-Met), CD31 and Ki67 in tumor sections were detected by IHC. The tumor tissues were harvested at the end of efficacy study following last dosing. Combination treatment displayed stronger inhibition on CD31 and Ki67, compared to either savolitinib or fruquintinib alone. **(c)**. Met signaling analysis in tumors by Western blot following a single oral dose of savolitinib, fruquintinib and their combination. Savolitinib inhibited p-Met in a time-dependent manner.

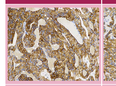
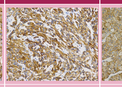
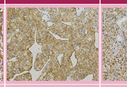
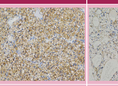
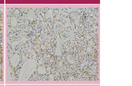


(d). Mice bearing established KIN1T1342 PDX model were treated orally with the daily indicated doses of savolitinib and fruquintinib (**, P<0.01). The increased anti-tumor effect was correlated with dose increment of savolitinib, indicating that enhanced anti-tumor effect was associated with c-Met inhibition. Combination treatment was well tolerated (no body weight loss). **(e)**. p-Met, CD31 and Ki67 in tumor sections were also analyzed by IHC. Combination treatment exhibited more potent inhibition on CD31 and Ki67, compared to either savolitinib or fruquintinib alone.



Anti-tumor efficacy studies were performed on other three established ccRCC CDX and PDX models: A498 (f), KIN1T1069 (g) and KIN1T1312 (h). *, P<0.05; **, P<0.01.

c-Met expression in 5 ccRCC xenograft models and anti-tumor efficacy summary

ccRCC model	A498	Caki-1	KIN1T1069P6	KIN1T1342P5	KIN1T1312P4
MET IHC					
IHC score	3+	3+	2+	2+	2+
H score	285 (3+,90%)	255 (3+, 65%)	230 (2+, 50%)	210 (2+, 50%)	180 (2+, 60%)
TGI of fruqu.	69	35	72	42	99
TGI of savol. @10 mpk	-	59	-	-	-
TGI of savol. @30 mpk	36	-	48	52	49
combination	114 (25% regression)	89	100	81	111 (23% regression)

TGI, tumor growth inhibition. Fruqu.: fruquintinib. Savol.: Savolitinib.

Summary

- c-Met expression was frequently detected in Chinese patients with ccRCC.
- Treatment with savolitinib or fruquintinib at clinically relevant doses resulted in moderate tumor growth inhibition as single agents in ccRCC xenograft models with high levels of c-Met expression. Significantly increased anti-tumor effect was observed in all models when the two agents were used in combination. In KIN1T1342, the anti-tumor effect was further increased with the increase of savolitinib dose possibly due to better target coverage.
- Consistent with more robust anti-tumor effect, the combination treatment produced stronger inhibition on tumor proliferation marker Ki67 and angiogenesis marker CD31, compared to either savolitinib or fruquntinib alone. These results indicated that the observed synergistic effect might be attributed to the dual inhibition on tumor signaling and tumor microenvironment.
- These results may highlight the potential clinical utility of savolitinib in combination with fruquintinib for the treatment of clear cell renal cell carcinoma with positive c-Met expression.

References

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4. Mazières J et al. Lung Cancer. 2013;82(2):231-7.