Preclinical characterization of CPL304110 as a potential selective inhibitor of Fibroblast Growth Factors 1/2/3 in solid cancers

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INTRODUCTION

Fibroblast Growth Factor Receptor (FGFR) family of receptor tyrosine kinases (RTK) contains four members – FGFR1, FGFR2, FGFR3, FGFR4. FGFRs play a critical role in cell proliferation, migration, angiogenesis and survival. Dysregulation of FGF/FGFR signaling pathway can result in formation of different tumour types including bladder, gastric, endometrial and lung cancer [1]. Therefore, targeting FGFRs represents an attractive strategy for anticancer therapy for patients with FGFR-dependent tumours [2]. Here we present CPL304110, a potent and selective small molecule that acts as FGFR 1/2/3 inhibitor and can be use as a potential drug for cancers with FGFRs aberrations.

MATERIALS AND METHODS

Compound cytotoxicity was assesed on various cancerous and control cell lines [Tab. 1a]. Two types of cell lines were used in all studies: one group FGFR dependent with confirmed genetic FGFR aberrations and second - FGFR independent. We also used normal cell line HUVEC as a control. All cell lines were cultured according to manufacturers' instructions and confirmed negative for mycoplasma (Venor®GeM qEP Mycoplasma Detection Kit for qPCR from Minerva Biolabs). Activity of CPL304110 was evaluated against FGFRs in ADP-based enzymatic test and its selectivity was tested on KINOMEscan® screening platform. The biological potency of compound was evaluated in a number of cancer cell-based models using Western Blot and cell viability assay (ATPlite™). Our inhibitor was also characterized in BioMAP Diversity PLUS panel. *In vivo* antitumour efficiency was verified on carcinoma xenograft models with FGFR1/2/3 aberrations: SNU-16, RT-112 and UM-UC-14. The studies were approved by Local Ethical Committee.

a

Cell line	Tissue	FGFR alteration
FGFR-dependent tumor cell lines		
NCI-H1581	lung	FGFR1 amplification FGFR3 mutation N549K
SNU-16	gastric	FGFR2 amplification
RT-112/84	bladder	FGFR3:TACC3 fusion FGFR3 amplification
KATO III	gastric	FGFR2 amplification
UM-UC-14	bladder	FGFR3 mutation S249C
AN3CA	uterus	FGFR2 amplification
KMS 11	myeloma	IgH-FGFR3 translocations
SW-780	bladder	FGFR3:BAIAP2L1 fusion
NCI-H520	lung	FGFR1 amplification
CAL120	breast	FGFR1 amplification

b

Cell line	Tissue
FGFR-independent tumor cell lines	
WSU-NHL	lymphoma
HEL 92.1.7	bone marrow
HepG2	liver
HCT116	colon
HeLa	uterus
U937	lymphoma
T24	bladder
LoVo	colon
H460	lung
PC-3	prostate
Normal cell line	
HUVEC	umbilical vein

TABLE 1. List of cell lines used in analysis.
a. FGFR-dependent tumor cell lines; b. FGFR-independent tumor cell lines and normal cell line.

STRATEGMED

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RESULTS

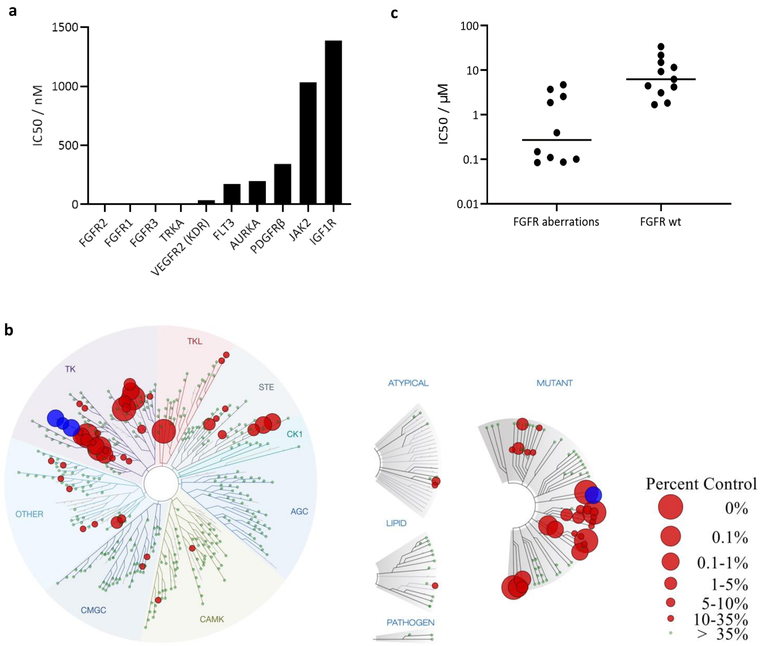

FIGURE 1. The activity and selectivity *in vitro* analysis.
a. *In vitro* inhibitory profile of CPL304110 based on ADP-Glo Kinase Assay. b. TREE*spot*™ Interaction Map for CPL304110 1000nM. Inhibited kinases with <35% of control were highlighted on the graph in red, while wild type or mutated FGFR1-3 receptors were highlighted in blue. c. IC_{50} values of CPL304110 in a human cell lines proliferation assay.

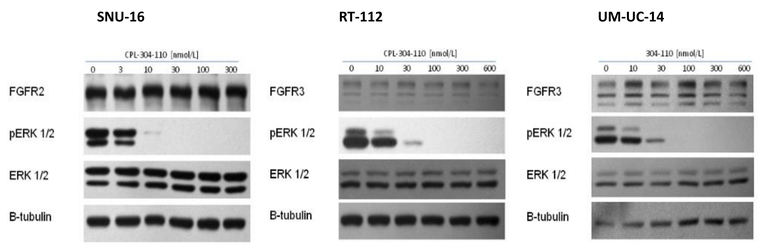

FIGURE 2. Cell lines with FGFR1/2/3 aberrations: SNU-16, RT-112 and UM-UC-14 treated with CPL304110 showed substantial decrease in activation of FGFR-mediated signaling.

RESULTS

First, potenial inhibitory effect on FGFR kinases was tested. Compound CPL304110 showed the strongest inhibitory activity determined as the IC_{50} on FGFR2 (IC_{50} = 1.44 nM), FGFR1 (IC_{50} = 4.08 nM) and FGFR3 (IC_{50} = 10.55 nM – highest value). In the next experiments CPL304110 selectivity was analyzed on 7 kinases, that display the highest structural homology to FGFR receptors (VEGFR2, PDGFRβ) or play important role in tumour development and progression (AURKA, FLT3, IGF1R, JAK2, TRKA). The highest inhibition value was determined for TRKA kinase (IC_{50} = 11nM) and KDR (IC_{50} = 37nM) [Fig. 1a].

As the final step, a broad *in vitro* screen against a panel of 468 kinases has been conducted for CPL304110. In this study 1 μM concentration of the compound has been used to determine potential offtargets. For FGFR1, FGFR2, FGFR3 and other kinases the calculated inhibition value are summarized in [Fig. 1b]. Kinome analysis included e.g. RET, FLT3, KIT and members of the VEGFR, PDGFR, and JAK families. Obtained data showned that CPL304110 was a potent FGFR1, FGFR2, FGF3 inhibitor but also has the strong inhibitory activity toward: CSF1R, FLT3(D835V), KIT(A829P), RET, RIPK1, TRKA, TYK2, KIT(V559D) and RET(M918T). The additional inhibitory effect may no only be beneficial, but also presenting CPL304110 as a promising agent for treatment of patients with cancers with specified mutations.

The antiproliferative activity of CPL304110 was determined in a panel of human tumor cell lines of different origin and FGFR dependant. The effect of CPL304110 on cell proliferation was also verified on the normal, non-neoplastic cell line – HUVEC to determine the potential safety margin. The results of the IC_{50} values are summarized in [Fig. 1c]. Cells treated with CPL304110 showed substantial decrease in activation of FGFR-mediated signaling [Fig. 2]. In the Diversity PLUS panel CPL304110 showed broad antiproliferative and antinflammatory impact. *In vivo*, oral administration of CPL304110 resulted in strong antitumour efficacy in all tested xenograft models [Fig. 3].

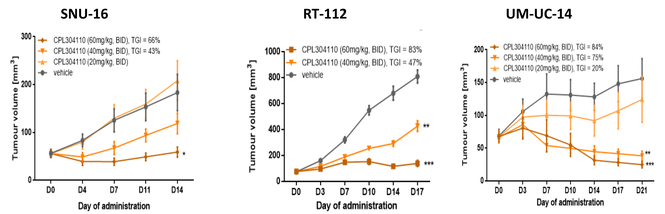

FIGURE 3. Efficacy of CPL303110 in different cancer cell line xenograft models with FGFR alterations.
SNU-16 gastric cancer model, RT-112 bladder cancer model, UM-UC-14 bladder cancer model. Data is presented as means +SEM for each group of mice (n=12 for A, C and n=6 mice for B); ANOVA post hoc Dunnett's versus vehicle-treated, *p < 0,05; **p < 0,01; ***p < 0,001. SEM, standard error of the mean. BID, twice a day.

CONCLUSIONS

Presented preclinical studies indicated that CPL304110 can be qualified as a good clinical candidate for treatment of FGFR-dependent tumours. Our drug is currently under Phase I clinical investigationl (01FGFR2018, NCT04149691).

REFERENCES

[1] Yun YR, et al. (2010). Fibroblast growth factors: biology, function, and application for tissue regeneration. Tissue Eng. 218142.
[2] Patani H, et al. (2016). Landscape of activating cancer mutations in FGFR kinases and their differential responses to inhibitors in clinical use. Oncotarget. 7(17):24252-68.