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

Delfina Popiel1, Agata Mikolajczyk1, Monika Skupińska1, Joanna Haruz-Kaltowska1, Paulina Starczak2, Natalia Piorokowska1, Abdellah Yamani1, Krzysztof Dubiel1, Aleksandra Starczak1, Jerzy Pieczykolan1, Maciej Wiecezorek1

1 Research and Development Center, Celon Pharma S.A, Kielce/Lomianki, Poland.

corresponding author: delfina.popiel@celonpharma.com; poster number #4126

INTRODUCTION

Fibroblast Growth Factor Receptor (FGFR) family of receptor tyrosine kinases (RTKs) 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 used as a potential drug for cancers with FGFRs aberrations.

MATERIALS AND METHODS

Compound cytotoxicity was assessed 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 alterations 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 (Vanguard™ Mycoplasma Detection Kit for qPCR from Minerva Biotechn). Activity of CPL304110 was evaluated against FGFRs in ADR-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 (ATPMs™). Our inhibitor was also characterized in BioMAP Diversity PLUS panel. In vivo antitumour efficiency was verified on carcinoma xenograft models with FGFR1/2/3 alterations: SNU-16, RT-112 and UM-UC-14. The studies were approved by Local Ethical Committee.

RESULTS

First, potential inhibitory effect on FGFR kinases was tested. Compound CPL304110 showed the strongest inhibitory activity determined as the IC50 on FGFR2 (IC50 = 0.2 μM), FGFR3 (IC50 = 4.8 μM) and FGFR3 (IC50 = 10.55 μM - highest value). In the next experiments CPL304110 selectivity was analyzed on 7 kinases, that display the highest structural homology to FGFR receptors (VEGFR2, POGFRB) or play important role in tumour development and progression (AKT/4, FLT3, FGFR1, JAK2, TRKA). The highest inhibition value was determined for TRKA kinase (IC50 = 11 μM) and KDR (IC50 = 37 μM) (Fig. 1a).

As the first step, a broad in vitro screening 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 off-targets. For FGFR1, FGFR2, FGFR3 and other kinases the calculated inhibition value are summarised in (Fig. 1b). Kinome analysis included e.g. RET, FLT3, KIT and members of the VEGFR, POGFR, and JAK families. Obtained data showed that CPL304110 was a potent FGFR1, FGFR2, FGFR3 inhibitor but also has the strong inhibitory activity toward: CSF1R, FLT3, Dop355; Kit738295, Ret, Hck, Y2k, TrkA, TrkY, Kit735800 and Ret999182. The additional inhibitory activity could be only 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 dependent. 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 IC50 values are summarised 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 antiflammatory impact. In vivo, oral administration of CPL304110 resulted in strong antitumour efficacy in all tested xenograft models (Fig. 3).

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.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue</th>
<th>FGFR alteration</th>
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<tbody>
<tr>
<td>NCI-H1581</td>
<td>lung</td>
<td>FGFR3 amplification</td>
</tr>
<tr>
<td>SN-12A</td>
<td>gastric</td>
<td>FGFR amplification</td>
</tr>
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<td>RT-112/B4</td>
<td>bladder</td>
<td>FGFR3/ACE5 fusion</td>
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<td>KAD-H11</td>
<td>gastric</td>
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<td>uterine</td>
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<td>bladder</td>
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<tr>
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</tr>
<tr>
<td>HUVEC</td>
<td>umbilical vein</td>
<td>normal</td>
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REFERENCES


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 investigation (01/FGFR2018, NCT04190961).

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