1960
PLEASE DO
NOT POST

CFON-026, A BEST-IN-CLASS MACROCYCLIC NON-COVALENT INHIBITOR OF BTK WT and BTK RESISTANCE MUTATIONS

Jos de Man, Michelle Muller, Joost C.M. Uitdehaag, Freek van Cauter, Sander P.W. van Gemert, Milan J. Hoffmann, Yvonne G.T.H. van Mil, Winfried R. Mulder, Martine B.W. Prinsen, Jan Gerard Sterrenburg, Diep Vu, Joeri J.P. de Wit, Erik Ensing, Rogier C. Buijsman.

Crossfire Oncology B.V., Kloosterstraat 9, 5349 AB Oss, the Netherlands. T: +31412700500 W: www.crossfire-oncology.com E: info@crossfire-oncology.com

Background

- Bruton Tyrosine Kinase (BTK) inhibitor drugs are a mainstay in the treatment of hematological cancers like CLL, MCL and WM.
- BTK inhibitor resistance occurs in 67% of relapsed CLL patients due to mutations such as BTK C481S, which abrogates covalent binding of acalabrutinib and ibrutinib [1].
- Pirtobrutinib is a non-covalent (reversible) BTK inhibitor drug that inhibits the BTK C481S mutation and is used as a second line therapeutic in CLL and MCL patients [2].
- However, >20% of patients on pirtobrutinib relapse because of emergence of a BTK T474I mutation (and to a lesser extent V416L and L528W) which impair binding of pirtobrutinib [2, 3].
- CFON-026 is a novel, best-in-class macrocyclic non-covalent BTK inhibitor of wild-type BTK and all clinically relevant BTK resistance mutations.

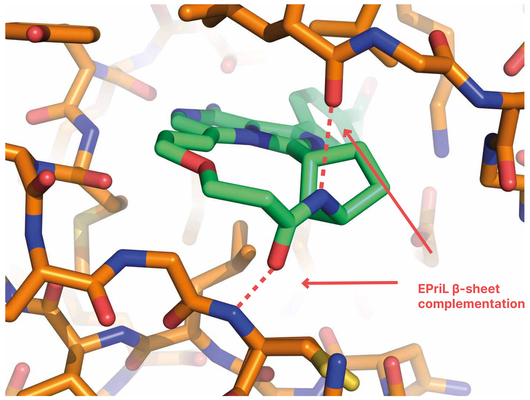

Figure 1: *X-ray structure of EPriL macrocycle binding in a kinase active site, showing that its tight binding is due to complementation of β-sheets in the kinase main chain, impeding the rapid emergence of resistance mutations.*

Methods

- At Crossfire Oncology we have developed a proprietary platform based on Energetically Privileged Ligands (EPriLs) [4].
- EPriLs are macrocycles designed using high-resolution X-ray structures of kinase-ligand complexes.
- The EPriL binding mode connects two parallel β-sheets in the general protein kinase protein fold conferring an intrinsic long residency to its target.
- EPriLs are used as inhibitors, degraders and in degrader-antibody conjugates [4].
- CFON-026 is an EPriL-based BTK inhibitor, selected for its best-in-class properties.

Results

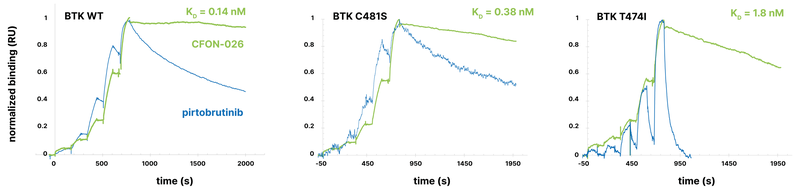

Figure 2: *Surface plasmon resonance shows long residence time of CFON-026 on BTK WT and clinically relevant resistance mutations BTK C481S and BTK T474I.*

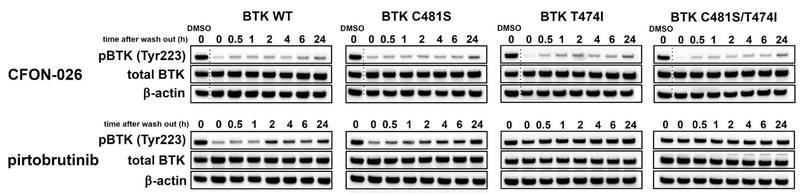

Figure 3: *CFON-026 (1 μM) shows more sustained inhibition of BTK signaling compared to pirtobrutinib (1 μM) in a wash-out experiment using HEK293 GripTite cells overexpressing BTK WT, BTK C481S, BTK T474I or BTK C481S/T474I.*

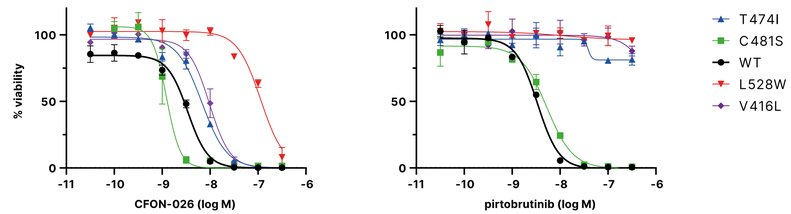

Figure 4: *Inhibition of proliferation using single cell clones of TMD8 BTK wild-type and TMD8 with endogenous BTK mutants (generated via CRISPR) demonstrates that CFON-026 inhibits all clinically relevant resistance mutations.*

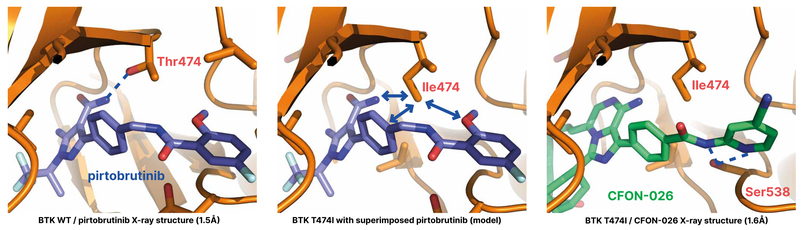

Figure 5: *Binding mode of CFON-026 and pirtobrutinib to BTK WT and BTK T474I . Pirtobrutinib binding is repulsed by BTK T474I. CFON-026 is able to bind BTK T474I because of its alternative contacts in the back pocket.*

Clinical Candidate Profile

BTK biochemical potency	
BTK WT (IC_{50}, IMAP)	0.27 nM
BTK C481S (IC_{50},IMAP)	0.19 nM
Target Residence Time ($1/k_{off}$) (BTK WT)	20,000 s
Selectivity (IC_{50} < 20·$IC_{50,BTK\ WT}$)	9 / 468 kinases

in vitro efficacy	
REC-1 (IC_{50})	1.7 nM
TMD8 (IC_{50})	0.45 nM
TMD8 mut (IC_{50}) C481S/T474I/V416L/L528W	1.1 / 6.7 / 9.7 / 114 nM
OCI-Ly3 (IC_{50})	> 20 μM

in vitro safety	
CYP inhibition (3A4, 2D6, 2C8, 2C9, 2C19, 1A2)	no inhibition < 1 μM
Eurofins safety panel (43 receptors/enzymes)	no binding

ADME	
HLM/RLM/MLM (μl/min/mg)	9.5 / 69 / < 3.5
Hepatocytes human/rat (μl/min/10^6cells)	12.0 / < 6.9
hPPB/mPPB (%)	98.6 / 98.6
hPlasma stability (%, 3h)	95
Caco2 P_{app} (10^{-6} cm/s), efflux ratio	18.7/ 0.7
$t_{1/2}$ (i.v.) mouse / dog (h)	1.2 / 4.5
Oral bioavailability mouse / dog (%)	42 / 34

in vivo efficacy	
TMD8 BTK WT xenograft	complete regression at 30 mg/kg

in vivo safety	
mouse multiple dose	no clinical signs observed; no treatment related effects on clinical chemistry and histopathology

Table 1: *CFON-026 clinical candidate profile. IND-enabling toxicology and manufacturing studies are currently ongoing.*

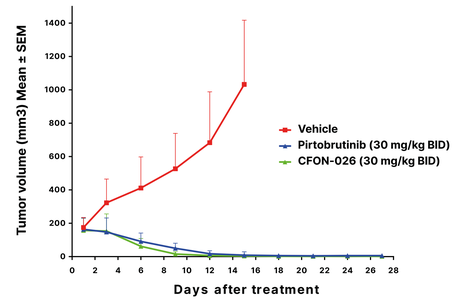

Figure 6: *CFON-026 shows complete regression of TMD8 BTK wild-type in a therapeutic TMD8 mouse model.*

Conclusions

- CFON-026 is a potent, highly selective and orally bioavailable macrocyclic non-covalent BTK inhibitor with best-in-class potential.
- CFON-026 shows slow dissociation kinetics resulting in a long target residence time on BTK WT and other resistance mutations BTK C481S and BTK T474I, leading to prolonged inhibition of BTK signaling.
- CFON-026 is, to our knowledge, the only non-covalent BTK inhibitor which inhibits all known clinically relevant BTK resistance mutations (BTK C481S, T474I, L528W, V416L).
- In a therapeutic TMD8 mouse *in vivo* model, complete tumor regression was observed.
- In line with the good tolerability in xenograft studies, no relevant findings from non-GLP 2-week toxicological studies in mice were reported.
- CFON-026 is currently progressing to IND filing.

References: [**1**] Woyach *et al.*, J Clin Oncol (2017). [**2**] Wang *et al.*, N. Eng J Med (2022). [**3**] ASH 2023 abstract no. 326. [4] AACR 2024 abstract nos. 6044 and 5814.