Preclinical Evaluation of CFT1946 as a Selective Degrader of Mutant BRAF for the Treatment of BRAF Driven Cancers


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Disclosure Information

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- I have the following financial relationships to disclose:
  - Stockholder in: C4 Therapeutics
  - Employee of: C4 Therapeutics
Mechanism of BRAF-V600X Driven Human Cancers

**NORMAL CONDITION**

- BRAF is a serine/threonine protein kinase in the MAPK pathway that promotes cell proliferation and survival when activated through extracellular signals.

**BRAF-V600X CONDITION**

- Constitutively active BRAF-V600X causes uncontrolled MAPK signaling, leading to tumorigenesis, tumor growth, and maintenance.
- Decreasing BRAF-V600X activity in these cancers leads to growth arrest, cell death, and tumor regression.
- BRAF-V600X is a clinically validated oncology target, however limitations in currently approved inhibitors highlight the need for additional BRAF-V600X targeted therapies.

MAPK, MAP kinase.
Utilizing a Degrader Approach to Overcome Limitations of BRAF Inhibition

**Degrader Rationale**

1. Application of Inhibitor Drug
2. Application of Degrader Drug
3. IRAS

**Advantages of BRAF V600X Degradation**

- Specifically target mutant BRAF-V600X over wildtype BRAF
- Prevent mutant BRAF-V600X incorporation into RAF dimers
- Avoid paradoxical activation of RAF dimers
- Address failures in inhibitor-based therapy due to resistance mechanisms
- Effect deep elimination of mutant BRAF signaling and create durable responses
CFT1946 is an On-Mechanism, CRBN-Based, BRAF-V600X BiDAC™ Degrader

CFT1946 Degrades BRAF-V600E in a Dose Dependent Manner

- HiBiT assay shows BRAF-V600E degradation with CFT1946 treatment in dose-dependent manner
- pERK loss aligns with loss of BRAF-V600E protein demonstrating MAPK pathway inhibition

\[ DC_{50} = 14 \text{ nM} \]
\[ E_{\text{max}} = 26\% \]

CFT1946 is an On-Mechanism BiDAC™ Degrader

- BRAF-V600E degradation with CFT1946
- No BRAF-V600E degradation with ligand competition, CRBN ligand competition, inhibition of CUL4 E3 with MLN4924 or inhibition of the proteasome with bortezomib

BiDAC, bifunctional degradation activating compound; HiBiT, high affinity bioluminescent tag; IMiD, immunomodulatory imide drug.
C4 Therapeutics data on file.
CFT1946 Degrades BRAF-V600E with No Activity on WT-BRAF, CRAF, or ARAF

Proteome Profiling Demonstrates Selectivity of CFT1946 for BRAF-V600E

CFT1946 (300 nM, 24 h) in A375 Cells

- Log10 p-value vs. dabrafenib

Log2 Fold Change vs. dabrafenib

8,760 proteins

BRAF-V600E

LOXL4

RAF1

ARAF

Proteome Profiling in WT-BRAF Cells Demonstrates Selectivity of CFT1946 for mBRAF

CFT1946 (300 nM, 24 h) in JURKAT Cells

- Log10 p-value vs. DMSO

Log2 Fold Change vs. DMSO

8,415 proteins

BRAF

RAF1

ARAF

C4 Therapeutics data on file.
CFT1946 Causes BRAF-V600E Degradation, Potent Inhibition of MAPK Signaling, & Loss of Viability in BRAF-V600E Cells but Not in WT-BRAF Cells

BRAF-V600E Degradation by CFT1946 Causes Loss of MAPK Signaling Superior to Inhibition Alone

CFT1946 Causes BRAF-V600E Degradation, Potent Inhibition of MAPK Signaling, & Loss of Viability in BRAF-V600E Cells but Not in WT-BRAF Cells

*note: CFT1946NMe is a non-CRBN binding version of CFT1946; BRAF is BRAF-V600E MAPK, MAP kinase.

C4 Therapeutics data on file.
CFT1946 Induces Tumor Regression in the BRAF-V600E A375 Xenograft Mouse Model in Accordance with PK/PD Results

CFT1946 Treatment of A375 Cell Line in vivo Shows Dose-Dependent Tumor Regression Superior to Inhibitor

Dose Proportional PK and PD for CFT1946

CFT1946 dose-response xenograft data demonstrates that 10 mg/kg BID dose results in sustained tumor regression and is the minimum efficacious dose.

BID, twice a day; MAPK, MAP kinase; PO, by mouth; PK/PD, pharmacokinetics/pharmacodynamics; QD, once daily.

C4 Therapeutics data on file.
CFT1946 is Active in BRAF-V600E/NRAS-Q61K, a Model of Clinical Resistance to BRAF Inhibitors

CFT1946 as a Single Agent and in Combination with MEKi is Effective in MAPK Pathway Inhibition, Superior to BRAFi

Combination Treatment of BRAFi Resistant Xenograft Model with CFT1946 and MEKi Shows Tumor Growth Inhibition/Regression

Figure: A375 + NRAS-Q61K Xenograft

- Vehicle (PO BID)
- MEKi: Trametinib (0.1 mg/kg BID)
- Encorafenib (35 mg/kg QD + MEKi)
- CFT1946 (10 mg/kg BID)
- CFT1946 (30 mg/kg BID)
- CFT1946 (10 mg/kg BID + MEKi)
- CFT1946 (30 mg/kg BID + MEKi)

BID, twice a day; BRAFi, BRAF inhibitor; MAPK, MAP kinase; MEKi, MEK inhibitor; PO, by mouth; PK/PD, pharmacokinetics/pharmacodynamics; QD, once daily.

C4 Therapeutics data on file.
CFT1946 Demonstrates Potential of TPD-Based Therapies in non-V600X mBRAF Driven Cancers

CFT1946 Provides PoC for Degradation of Selected non-V600E mBRAF of Both Class II and Class III

Using an ectopic expression system in HEK293T cells, CFT1946 treatment demonstrates degradation of HA-tagged mBRAF in a dose-dependent manner.

CFT1946 Treatment of Class III mBRAF Model Cell Line Shows PoC for TPD-mediated Growth Inhibition Superior to BRAFi

CFT1946 treatment of H1666 cells shows modest growth inhibition, superior to inhibition alone.

BRAFi, BRAF inhibitor; MEKi, MEK inhibitor; PoC, proof of concept; TPD, targeted protein degradation.

C4 Therapeutics data on file.
CFT1946 is a potent and mutant-selective BiDAC™ degrader of BRAF-V600X

CFT1946 is active *in vitro* and *in vivo* in models with BRAF-V600E–driven disease and in the escape mutant BRAF-V600E/NRAS-Q61K–driven model

CFT1946 demonstrates that a TPD approach could be developed to address mBRAF Class II and Class III driven cancers

CFT1946’s preclinical profile warrants clinical evaluation in patients with both BRAF-V600X–driven cancers and inhibitor-resistant BRAF-V600X–driven cancers
Acknowledgments

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