Abstract
#233

CFT7455: A novel, IKZF1/3 Degrader That Demonstrates Potent Tumor Regression in a Spectrum of Non-Hodgkin Lymphoma Xenograft Models

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Introduction

Introduction: Ikaros family zinc finger protein 1 and 3 (IKZF1/3) are essential transcription factors (TF) for differentiation of B and T lymphocytes. IMiDs (e.g. pomalidomide (pom)) degrade IKZF1/3 via interaction with the cereblon (CRBN) E3 ligase and have shown promise in NHL. Preclinical data suggest improvements in IKZF1/3 degraders may lead to enhanced efficacy. CFT7455 is a novel IKZF1/3 degrader optimized for high affinity CRBN binding and IKZF1/3 degradation, resulting in downregulation of the interferon regulatory factor 4 (IRF4), a critical regulator in non-Hodgkin's lymphoma (NHL), including diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL) and peripheral T-cell lymphoma (PTCL).

Methods: Protein expression in cell lines and tumor xenografts were quantified by immunoassays or targeted mass spectrometry. Binding affinity was determined by fluorescence polarization or cellular NanoBRET assays. Cell viability were monitored by CellTiter-Glo. Tumor xenograft studies were conducted by implanting human NHL lines into immunocompromised mouse strains.

Results: CFT7455's potency as a CRBN binder is evident from cellular CRBN competition studies (IC50 = 0.4 nM). In the ALK+ anaplastic large cell lymphoma (ALCL) line KiJK, CFT7455 treatment led to >80% degradation of IKZF1, which was blocked by proteasome or NEDD8 inhibition, demonstrating on-mechanism activity. CFT7455 demonstrated potent antiproliferative activity across a panel of NHL cell lines.

In KiJK xenografts, pom treatment was ineffective at a clinically relevant dose (3000 µg/kg/day). CFT7455 treatment (100 µg/kg/day, PO) resulted in durable tumor regression associated with deep IKZF3 degradation and IRF4 downregulation (7% and 25% remaining, respectively). CFT7455 showed dose dependent efficacy in the ALK-ALCL xenograft model, DL40, from 3-100 µg/kg with regressions at doses ≥ 10 µg/kg. Global proteomic studies on DL40 xenografts treated with CFT7455 (100 µg/kg, 4 hours) showed only IKZF1/3 were significantly degraded.

MCL is characterized by elevated cyclin D1, subsequent release of E2F1 and pathway activation. In the REC1 MCL xenograft model, doses of CFT7455 ≥ 10 µg/kg promoted tumor regression. Pharmacodynamic studies showed that CFT7455 (30 µg/kg) promoted degradation of IKZF3 and downregulation of cyclin D1 and E2F1.

In a pom insensitive DLBCL model (TMD8), administration of CFT7455 (100 µg/kg) led to tumor regression. Together these results show that the optimized CRBN binding and catalytic activity of CFT7455 results in rapid, deep and sustained degradation of IKZF1/3 and translates to tumor regressions in NHL models.

Conclusions: CFT7455 is a potent, selective catalytic degrader of IKZF1/3, with single agent antitumor activity in DLBCL, ALCL, and MCL models including those insensitive to pom. These results support clinical investigation of CFT7455 for NHL.

CFT7455 is Positioned to Address IKZF1 (Ikaros) and IKZF3 (Aiolos) Dependent Lymphomas

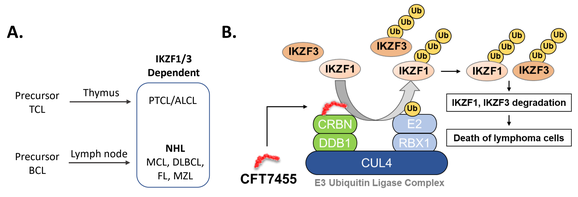

Figure 1. IKZF1/3 dependent lymphomas and mechanism of action for CFT7455
Ikaros family zinc finger protein 1 and 3 (IKZF1/3) are essential transcription factors for differentiation of T and B lymphocytes. Modulation of IKZF1/3 protein expression, genetically or by small molecule degraders, have demonstrated dependencies and/or clinical activity in peripheral T cell lymphomas[1,2] (PTCL) and NHL including mantle cell lymphoma[3], diffuse large B cell lymphoma[4], follicular lymphoma[5], and marginal zone lymphoma[6]. CFT7455 is a novel, small molecule anticancer agent that binds with high affinity to the CRBN E3 ligase, creating a new surface on CRBN for interaction with IKZF1/3 (Figure 1B). As a result, IKZF1/3 are ubiquitinated by the CRBN E3 ligase and degraded by the proteasome, resulting in the death of cells dependent on IKZF1/3, including subsets of lymphoma and multiple myeloma.

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CFT7455 Displays High Affinity CRBN Binding and Potent, Deep IKZF1 Degradation

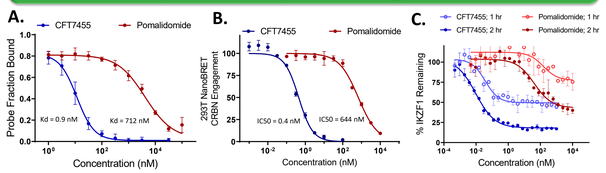

Figure 2: Binding properties and in vitro degradation kinetics for CFT7455 and pomalidomide

A. Binding affinity of CFT7455 to CRBN-DDB1 is ~800X more potent than pomalidomide. Binding of CFT7455 and pomalidomide to CRBN-DDB1 was measured by fluorescent polarization.

B. CFT7455 potently displaces pomalidomide bound to CRBN in cells. CRBN binding was measured by displacement of a pomalidomide-NanoBRET fluorescent tracer in 293T cells expressing cereblon fused to NanoLuc® (CRBN-NanoLuc).

C. CFT7455 induces rapid and deep IKZF1 degradation. IKZF1 degradation was measured in NCIH929 myeloma cells modified to express HiBiT-tagged IKZF1 using Nano-Glo HiBiT Lytic Assay System.

CFT7455 Demonstrates Potent Antiproliferative Activity in PTCL, DLBCL and MCL Cell Lines

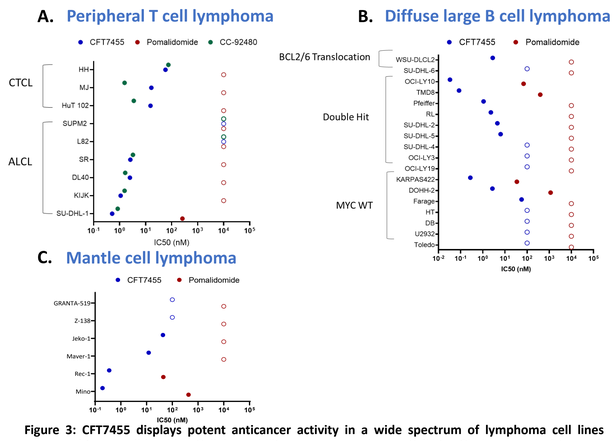

Figure 3: CFT7455 displays potent anticancer activity in a wide spectrum of lymphoma cell lines compared to pomalidomide. Cellular viability was measured using CellTiter Glo in a panel of NHL cell lines treated with compounds *in vitro* for 96 hr. Open symbols indicate that growth was not inhibited by more than 50% at the highest tested concentration (100 nM or 10 µM for CFT7455, 10 µM for pomalidomide, and 10 µM for CC-92480) and therefore IC50 was not determined.

A. Anticancer activity of CFT7455 in PTCL. Graphs are divided to show CTCL (cutaneous T-cell lymphoma) and ALCL lineages.

B. Anticancer activity of CFT7455 in DLBCL DLBCL cell lines are arranged by MYC status. Double hit = MYC and either BCL2 or BCL6. Status determined from CCLE, CBioPortal, and literature.

C. Anticancer activity of CFT7455 in MCL.

CFT7455 Promotes Selective Degradation of IKZF1/3, Resulting in Modulation of IFN Regulated Genes

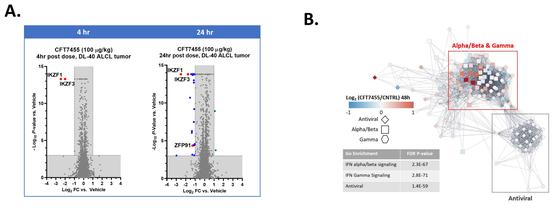

Figure 4. Proteome profiling of CFT7455 in ALCL xenograft tumors

A. CFT7455 selectively degrades IKZF1 and IKZF3 in ALCL tumors. DL-40 ALCL xenograft tumors were isolated from mice 4 and 24 hours after oral administration of 100 µg/kg CFT7455 or vehicle. Tumors were lysed, reduced/alkylated, digested and labeled with isobaric TMT-10 reagents. A mixed sample was fractionated, and consolidated fractions were analyzed with LC-MS/MS. A total of 7,903 proteins were quantified. Scatter plots depict the fold change in relative abundance of proteins from CFT7455 treated mice compared to vehicle treated mice. Negative Log_{10} adjusted P values are shown on the *y* axis and Log_2 fold changes are on the *x* axis.

B. Primary actions of CFT7455 are on Interferon-response genes. DL-40 ALCL xenograft tumors were isolated from 48 hours after oral administration of 100 µg/kg CFT7455 or vehicle. Gene ontology analysis was performed and the interactions among altered proteins were determined using the STRING database, with a cutoff score of 700. The resulting interactions were visualized with Cytoscape. Nodes are colored according to their down/up-regulation by CFT7455 compared to vehicle. Interferon (IFN) signaling was the most prominent network impacted by CFT7455.

CFT7455 is More Potent and Efficacious Than Pomalidomide or CC-92480 in ALCL Tumor Models

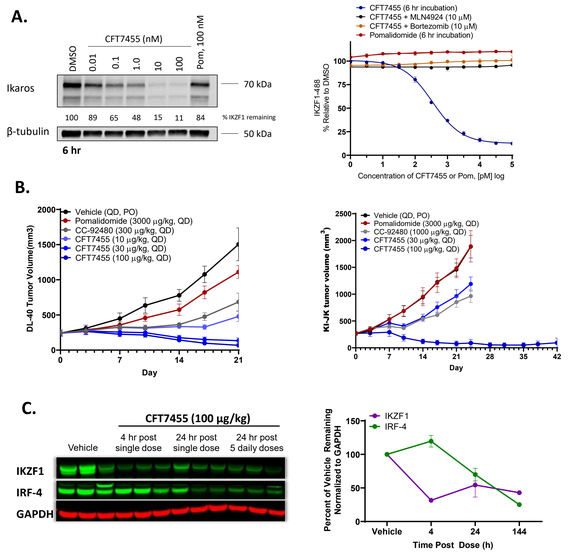

Figure 5: In vitro and in vivo activity of CFT7455 in ALCL tumor models

A. Degradation of IKZF1 in ALCL models by CFT7455 is CRBN dependent. KI-JK cells were treated for 6 hours with CFT7455 or pomalidomide (Pom) as indicated. IKZF1 was detected by western blot (left) or flow cytometry (right). Mechanism of action was confirmed by co-treating cells with CFT7455 and bortezomib (proteasome inhibitor) or MLN-4924 (NEDD8 activating enzyme inhibitor).

B. CFT7455 displays superior efficacy in 2 ALCL xenograft tumor models compared to pomalidomide and CC-92480. Mice bearing established DL-40 or KI-JK xenografts were administered pomalidomide (3000 µg/kg/day), CC-92480 (300 or 1000 µg/kg/day), or CFT7455 (10, 30 or 100 µg/kg/day) on a daily PO regimen for 21 days. Data are expressed as mean tumor volumes ± SEM.

C. CFT7455 treatment leads to deep and durable IKZF1 degradation and IRF4 suppression. Mice bearing established KI-JK xenografts were administered a single dose CFT7455 (100 µg/kg) or daily for 5 days. Tumors were collected at 4 and 24 hours post single dose and 24 hours post 5 daily doses. Tumors were analyzed by western blot for IKZF1 and IRF-4 levels. Quantitation is represented as percent of vehicle remaining normalized to GAPDH.

CFT7455 Triggers Caspase-Mediated DLBCL Cell Death and is Efficacious in Pom-Insensitive Model

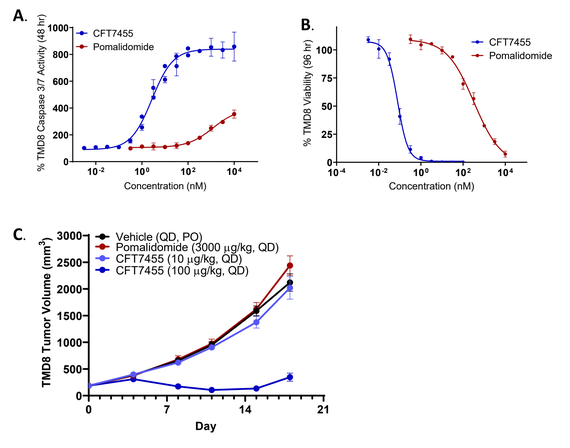

Figure 6: In vitro and in vivo activity of CFT7455 in the TMD8 DLBCL model

A. Caspase activation in DLBCL models is dose dependent. Caspase 3/7 activity was measured by cleavage of luminogenic substrate (Promega) in TMD8 cells treated with compounds *in vitro* for 48 hr and normalized to DMSO-treated controls.

B. CFT7455 demonstrates potent antiproliferative activity in DLBCL. Cellular viability was measured using CellTiter Glo in TMD8 cells treated with compounds *in vitro* for 96 hr.

C. CFT7455 promotes regressions in DLBCL xenograft model. Mice bearing established TMD8 xenografts were administered pomalidomide (3000 µg/kg/day) or CFT7455 (10 or 100 µg/kg/day) on a daily PO regimen for 17 days. Data are expressed as mean tumor volumes ± SEM.

CFT7455 Effectively Degrades IKZF1 Leading to Downregulation of IRF-4, Cyclin D1 and E2F1 in MCL model

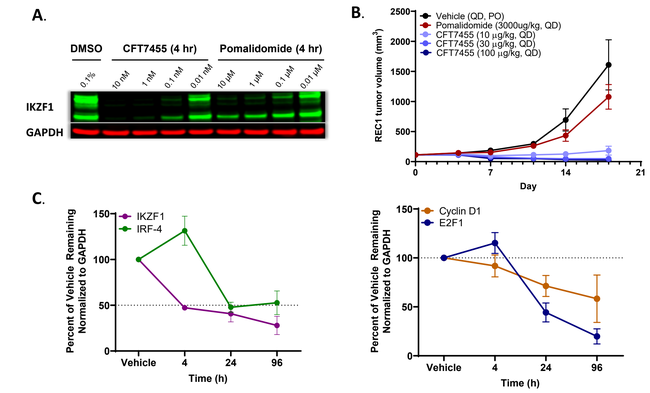

Figure 7: CFT7455 degrades IKZF1 leading to reduced IRF-4, E2F1 and Cyclin D1 in a MCL xenograft model

A. CFT7455 demonstrates improved potency and IKZF1 degradation in MCL cells compared to pomalidomide. REC-1 cells were treated with a dose response of CFT7455 (10 nM to 0.01nM) or pomalidomide (10 µM to 0.01 µM) for 4 hours. Cells were analyzed by western blot for IKZF1 and GAPDH levels.

B. CFT7455 promotes durable regressions in MCL xenograft model. Mice bearing established REC-1 xenografts were administered pomalidomide (3000 µg/kg/day) or CFT7455 (30 or 100 µg/kg/day) on a daily PO regimen for 19 days. Data are expressed as mean tumor volumes ± SEM.

C. Downregulation of Cyclin D1 and E2F1 in MCL xenograft model post CFT7455 treatment. Mice bearing established REC-1 xenografts were administered CFT7455 (30 µg/kg) for 3 days. Tumors were collected at 4 and 24 hours post single dose and 24 hours post 3 daily doses. Tumors were analyzed by western blot for IKZF1, IRF-4, Cyclin D1, and E2F1. Quantitation is represented as percent of vehicle remaining normalized to GAPDH.

Summary

- CFT7455 is a highly potent CRBN binder that promotes the selective degradation of IKZF1 and IKZF3 in preclinical NHL models
  - CRBN binding affinity: Kd 0.4-0.9 nM
  - Degradation response: 80% IKZF1 degradation by 2 hr at 1 nM
  - Selectivity: Only IKZF1 and IKZF3 significantly reduced in ALCL tumors from >7900 proteins detected
  - Cellular activity: CTCL, ALCL, MCL, and high-grade B-Cell lymphoma with MYC, BCL2, and/or BCL6 translocations/rearrangements
- CFT7455 exemplifies improved potency and efficacy in vivo compared to approved and investigational IMiDs
  - CFT7455 is 30X more potent than CC-92480 and >100X pomalidomide in ALCL tumor models
  - Deep, prolonged IKZF1 degradation enabled durable tumor regression in ALCL, DLBCL and MCL tumor xenograft models
- These encouraging data support the initiation of a first-in-human clinical trial to assess the safety and tolerability of CFT7455 in patients with R/R NHL (NCT04756726)

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