CFT7455: A novel IKZF1/3 degrader, demonstrates potent anti-tumor activity in peripheral and CNS models of non-Hodgkin's lymphoma as a single agent or in combination with clinically approved agents

Abstract # 411

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Introduction

Immunomodulatory drugs, such as pomalidomide and lenalidomide are approved for the treatment of multiple subtypes of NHL. These drugs induce an interaction between Ikaros family zinc finger proteins 1 and 3 (IKZF1/3) and cereblon (CRBN), an E3 ligase, resulting in IKZF1/3 degradation. IKZF1/3 are master transcription factors that are essential for viability of non-Hodgkin's lymphoma (NHL). Clinically, immunomodulatory drugs are active as single agents and when used in combination with several classes of targeted therapies, in both first line and relapsed/refractory NHL subtypes.

We previously described the preclinical characterization of CFT7455 as a novel IKZF1/3 degrader with 800-1600-fold increased potency over pomalidomide in CRBN binding and cellular IKZF1 degradation assays. We demonstrated that this increased activity translates into dramatically greater efficacy compared to pomalidomide in cellular and *in vivo* preclinical models of multiple myeloma and NHL. Here we show that the increased catalytic activity and improved potency of CFT7455 also translates into potent anti-tumor activity as a single agent or in combination with clinically approved agents in multiple models of NHL. In both the Raji and OCI-Ly10 CNS xenograft models, CFT7455 (100 µg/kg/day) led to a significant increase in survival probability in comparison to pomalidomide (3000 µg/kg/day) (P=0.002, P=0.0002).

The combination of immunomodulatory drugs with CD20, BTK, or HDAC targeting agents shows promising clinical activity in the treatment of NHL. Combination studies were conducted in which CFT7455 was dosed together with rituximab (anti-CD20 antibody), ibrutinib (BTK inhibitor), or romidepsin (HDAC inhibitor) in several models of NHL. In the mantle cell lymphoma (MCL) model, Mino, the combination of CFT7455 with rituximab demonstrated enhanced activity and complete tumor regression. In the diffuse large B-cell lymphoma (DLBCL) model, TMD8, the combination of ibrutinib and CFT7455 was synergistic and resulted in a significant increase in survival probability. Synergistic activity was also observed with the combination of CFT7455 and romidepsin in two models of ALCL. In the intracranial NHL model, OCI-Ly10, the combination of CFT7455 with rituximab or ibrutinib also led to a significant increase in survival probability, highlighting the therapeutic potential of this combination in CNS disease. In conclusion, CFT7455 is a potent, selective catalytic degrader of IKZF1/3, with single agent and combination antitumor activity in DLBCL, ALCL, and MCL, supporting clinical investigation of CFT7455 for NHL.

CFT7455 Targets IKZF1 (Ikaros) and IKZF3 (Aiolos) Dependent Lymphomas

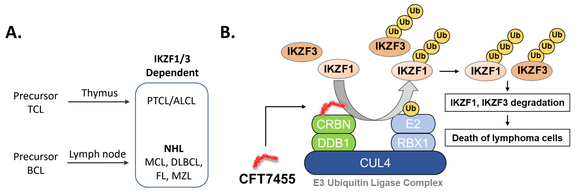

Figure 1. IKZF1/3 dependent lymphomas and mechanism of action for CFT7455
IKZF1/3 are essential transcription factors for differentiation of T and B lymphocytes. Modulation of IKZF1/3 protein expression, genetically or by small molecule degraders, have demonstrated dependencies and/or clinical activity 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.

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

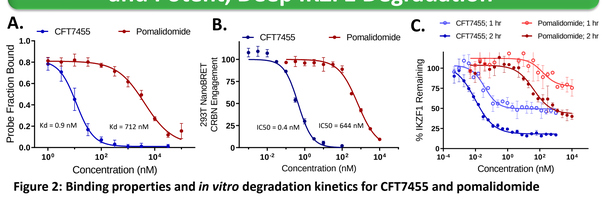

Figure 2: Binding properties and *in vitro* degradation kinetics for CFT7455 and pomalidomide

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

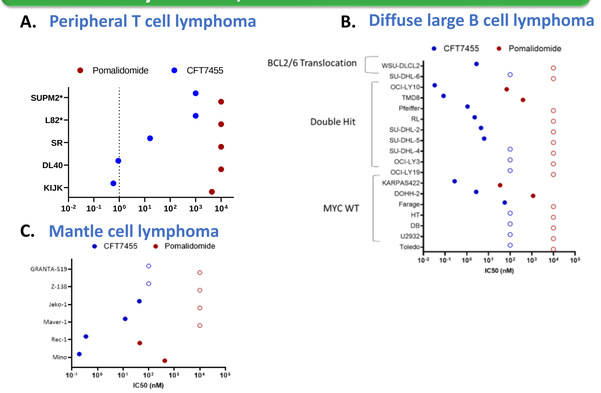

Figure 3: CFT7455 displays potent anticancer activity in a wide spectrum of lymphoma cell lines compared to pomalidomide. Cellular viability was measured using CellTiter Glo in a panel of NHL cell lines treated with compounds *in vitro* for 96 hr. Open symbols indicate that growth was not inhibited by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

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

***B. In vitro* 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. In vitro* activity of CFT7455 in MCL.**

CFT7455 Shows Benefit in Combination with Ibrutinib in the TMD8 DLBCL Model

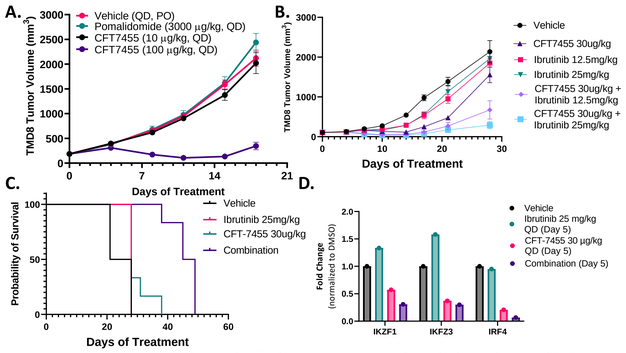

Figure 4: CFT7455 combination with ibrutinib shows synergistic activity in the TMD8 DLBCL model. Low dose CFT7455 (30 µg/mk QD), 12.5 or 30 mg/kg QD ibrutinib treatment led to modest tumor growth inhibition as single agents in the TMD8 model of DLBCL. Combination of the two agents induces tumor regression.

A. CFT7455 promotes regressions in a 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.

B. CFT7455 enhances the activity of ibrutinib in a DLBCL model. Mice bearing established TMD8 xenografts were administered CFT7455 (30 µg/kg/day) on a daily PO regimen and/or ibrutinib (12.5 mg/kg or 25 mg/kg) for 28 days. Data are expressed as mean tumor volumes ± SEM.

C. CFT7455 combined with ibrutinib increases survival probability of animals with DLBCL xenografts. Mice bearing established TMD8 xenografts were administered CFT7455 (30 µg/kg/day) on a daily PO regimen and/or ibrutinib (12.5 mg/kg or 25 mg/kg) for up to 54 days. Data are expressed as a Kaplan-Meier survival curve

D. PD analysis of TMD8 cells treated with CFT7455 and ibrutinib. Protein was isolated from the xenograft study described in Fig 4B and evaluated for IKZF1, IKZF3 and IRF4 expression by western blot.

CFT7455 Shows Benefit in Combination with Rituximab in the MCL Model, Mino

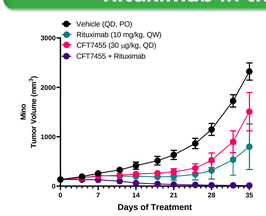

Figure 5. CFT7455 enhances the activity of rituximab in the MCL model, Mino. Mice bearing established Mino xenografts were administered CFT7455 (30 µg/kg/day) on a daily PO regimen and/or rituximab (10 mg/kg QW) for 35 days. Data are expressed as mean tumor volumes ± SEM.

CFT7455 Shows Benefit in Combination with Romidepsin in PTCL Models

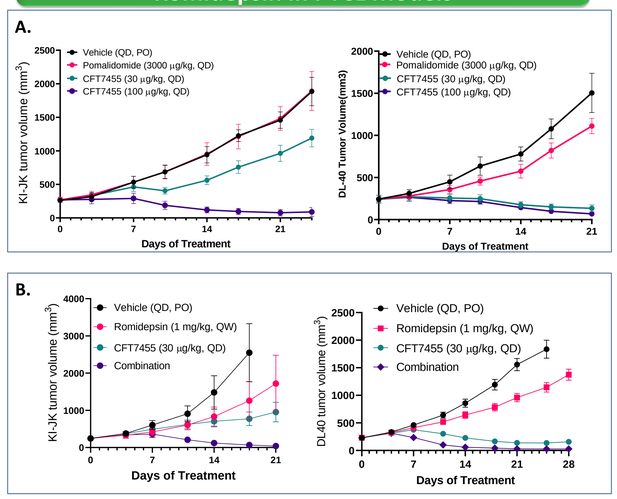

Figure 6: *In vivo* activity of CFT7455 in ALCL tumor models. CFT7455 shows single agent activity in KI-JK and DL-40 xenograft models. Treatment with low doses of CFT7455 in combination with romidepsin, an HDAC inhibitor, leads to tumor regression.

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

B. CFT7455 improves response to romidepsin in ALCL xenograft tumor models. Mice bearing established DL-40 or KI-JK xenografts were administered CFT7455 (30 µg/kg/day QD/PO), romidepsin (1 mg/kg QW/PO) or a combination of the two for 21 days. Data are expressed as mean tumor volumes ± SEM.

CFT7455 is Effective in a CNS Model of NHL

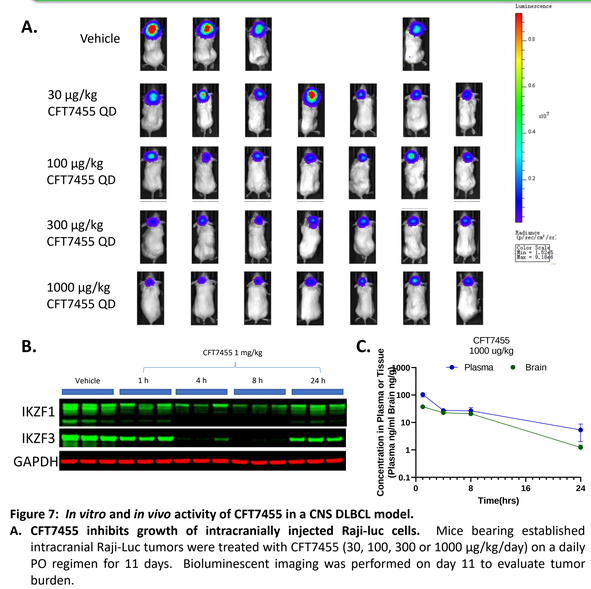

Figure 7: *In vitro* and *in vivo* activity of CFT7455 in a CNS DLBCL model.

A. CFT7455 inhibits growth of intracranially injected Raji-luc cells. Mice bearing established intracranial Raji-Luc tumors were treated with CFT7455 (30, 100, 300 or 1000 µg/kg/day) on a daily PO regimen for 11 days. Bioluminescent imaging was performed on day 11 to evaluate tumor burden.

B. CFT7455 degrades IKZF1/3 in intracranial Raji-luc tumors. Mice bearing established right hemisphere intracranial Raji-Luc tumors were treated with CFT7455 (1000 µg/kg/day) on a daily PO regimen for 1, 4 or 8, 24 hours. Tumors were isolated and evaluated for IKZF1 and IKZF3 levels by western blot.

C. CFT7455 is present in the CNS at active levels. Mice bearing established right hemisphere intracranial Raji-Luc tumors were treated with CFT7455 (1000 µg/kg/day) on a daily PO regimen for 1, 4 or 8 hours. Brain tissue from left hemisphere was isolated and evaluated by LC-MS for CFT7455 levels

CFT7455 Treatment Shows Improved Survival in a CNS DLCBL Model in Combination with Ibrutinib or Rituximab

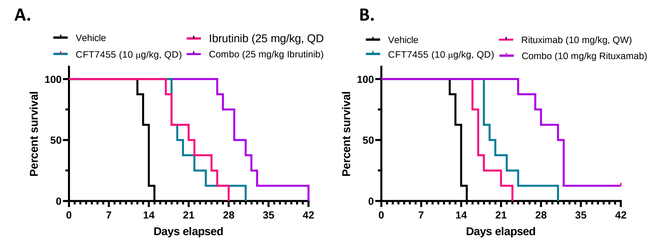

Figure 8. CFT7455 treatment shows increased survival probability in a CNS model of DLBCL, OCI-LY-10 as a single agent or in combination with ibrutinib or rituximab. Low dose CFT7455 (10 µg/kg QD) enhances survival of animals with intracranial OCI-LY10 tumors treated with ibrutinib or rituximab.

A. Kaplan-Meier survival curves of four animal cohorts bearing intracranial OCI-LY-10 xenografts following vehicle, CFT7455 (10 µg/kg QD), ibrutinib (25 mg/kg QD) or CFT7455 and ibrutinib combination treatments. CFT7455 + ibrutinib showed a 121.4% increase in median survival time (p<0.001).

B. Kaplan-Meier survival curves of four animal cohorts bearing intracranial OCI-LY-10 xenografts following vehicle, CFT7455 (10 µg/kg QD), rituximab (10 mg/kg QD) or CFT7455 and rituximab combination treatments. CFT7455 + rituximab showed a 92.9% increase in median survival time (p<0.001).in

Summary

- CFT7455 is a potent IKZF1/3 degrader currently being investigated in NHL in a Phase 1/2 clinical trial (NCT04756726)
- Models indicate CFT7455 may provide significant advantages over currently approved IKZF1/3 degraders in the treatment of NHL
  - CFT7455 has been shown to have potent activity as a single agent in models of NHL, including CTCL, ALCL, MCL and DLBCL[7]
  - CFT7455 demonstrates >100X activity *in vivo* than the clinically approved IKZF1/3 degrader pomalidomide in multiple NHL models[7]
  - Pharmacokinetic and potency profiles of CFT7455 allow prolonged degradation of IKZF1/3, leading to enhanced activity[7]
- In addition to single agent activity, in models, CFT7455 shows synergistic combination activity with approved agents in NHL
  - Combination of low dose of CFT7455 with the BTK inhibitor ibrutinib achieves tumor regression in the TMD8 model of ABC-DLBCL.
  - Combination of a low dose of CFT7455 with the CD20 monoclonal antibody rituximab achieves tumor regression in the Mino model of MCL
  - Combination of a low dose of CFT7455 with the HDAC inhibitor romidepsin achieves tumor regression in KI-JK and DL-40 models of ALCL
- CFT7455 shows activity in CNS models of lymphoma
  - Tumor growth inhibition and IKZF1/3 degradation were observed in intracranially implanted Raji-Luc tumors
  - CNS-implanted OCI-LY10 DLCBL model showed improved survival with CFT7455 as a single agent or in combination with ibrutinib or rituximab

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