Lessons learned in developing JAK2 degraders

Linda Lee[1], Ryan Michael[1], Roman Agafonov[1], Adam Crystal[1], Scott Eron[1], William Austin Elam[1], Mark Fitzgerald[1], Ashley Hart[1], Alex Hird[1], Jonathan Lewis[2], Tobie Lee[1], Michael Palmer[1], Eunice Park[1], Joe Patel[1], Andrew J. Philips[1], David Stokoe[2], Jennifer Tsai[1], Ellen Vieux[1], Rhamy Zeid[1], Robert Cohen[2], and Stew Fisher[1]

[1]C4 Therapeutics, Watertown, MA [2]Calico Life Sciences LLC, South San Francisco, CA

Introduction

Janus kinase 2, or JAK2, is a non-receptor tyrosine kinase that is involved in various processes such as cell growth, development, differentiation and histone modifications.[1] JAK2 consists of four domains: an active tyrosine kinase domain, the JAK homology 1 (JH1); a catalytically inactive pseudokinase domain, the JAK homology 2 (JH2); a SRC homology 2 domain (SH2); and an amino terminal FERM (4-point-1, Erzin, Radixin, Moesin) homology domain.[2] A somatic mutation located in the JH2 domain, V617F, is the most common mutation found in patients with myeloproliferative neoplasms (MPNs), including 95% of patients with polycythemia vera (PV), 60% of patients with essential thrombocythemia (ET), and 50% of patients with primary myelofibrosis (PMF).[3] The location of the V617F JAK2 mutation within the JH2 domain has made it difficult to target it with known inhibitors that typically bind to the JH1 domain. Inhibition of wild-type JAK2 results in toxic side effects, but the close homology between wild-type JAK2 and the mutant V617F protein presents a significant selectivity challenge.[4] We reasoned this selectivity challenge can be overcome via targeted protein degradation, where the selective degradation of mutant JAK2 can be achieved by leveraging the ubiquitin-proteasome system (UPS), especially the unique interactions between mutant JAK2 and the E3 ligase.

To explore this hypothesis, we synthesized a matrix-based library of JAK2 BiDAC degraders (Bifunctional Degradation Activating Compounds), based on six unique JAK2 ligands conjugated to binding warheads for the E3 ligases CRBN and VHL. This library incorporated multiple linker lengths and individual vectors comprising over 500 compounds. Screening in a HiBiT-tagged JAK2 V617F degradation assay led to the discovery of three cereblon-based putative hits from three different chemical series. To confirm these compounds were on mechanism, we undertook a series of experiments. Matched pair analogs, with modifications known to eliminate cereblon engagement, maintained JAK2 degradation activity, implying an off-target mechanism and thus eliminating one series of the initial hits from further consideration. The remaining two series demonstrated rescue of JAK2 levels when profiled using competition experiments with known cereblon ligands, the proteasomal inhibitor Bortezomib and the neddylation inhibitor MLN4924, indicating a cereblon-dependent degradation mechanism. In contrast, competition studies with JAK2 ligands did not rescue JAK2 degradation but surprisingly demonstrated improved potency. This result was attributed to inhibition of P-glycoprotein (PGP) by the JAK2 ligands and was confirmed by the PGP inhibitor Verapamil, in addition to the lack of the potency enhancement in cell lines known to have low to very little PGP expression. Finally, these putative hits were found to be positive in a counter screen against transcription termination factor GSPT1, a previously reported neo-substrate of cereblon. Taken together, these results demonstrate that the observed JAK2 degradation profile for the remaining two hit series was attributed to cereblon-mediated off-target degradation of GSPT1.

This library screen demonstrates the importance of including GSPT1 counter screens and evaluation of PGP substrate potential in addition to on-mechanism degradation profiling approaches to ensure bona fide degrader hit confirmation.

JAK2 BiDAC Degraders Matrix Library Design

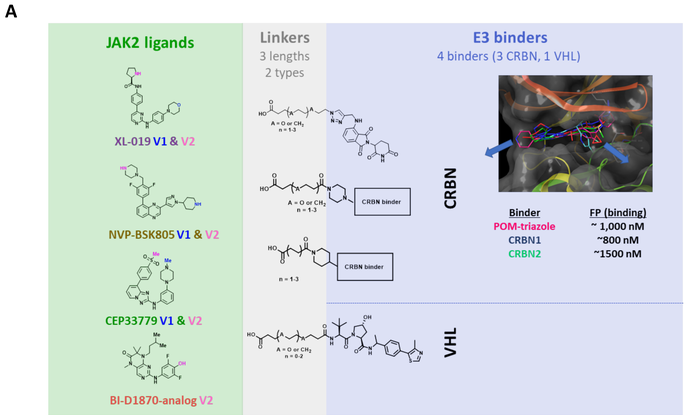
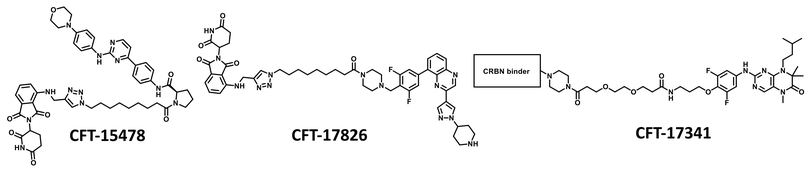


Figure 1: A Matrix JAK2 BiDAC libraries generated utilizing CRBN & VHL binders **B.** Library screening scheme

Three Putative Hits Identified That Induce >50% JAK2 V617F Loss in a HEL9217-based HiBiT Screen



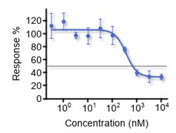
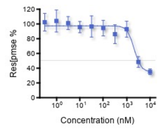
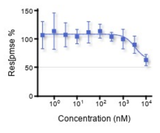
	JH1 binder-based		JH2 binder-based
	CFT-15478	CFT-17826	CFT-17341
JAK2 V617F degradation (24 h)	DC50=880nM, Emax=43% (n=16)	DC50=3624nM, Emax=34% (n=3)	DC50=5562nM, Emax=42% (n=2)
			

Figure 2: HiBiT-detection of JAK2 with increasing concentration of JAK2 BiDAC degrader using HEL9217 cells with endogenously tagged JAK2 [V617F]. Bars represent SD.

UPS-dependent Mechanism Confirmed for CFT-15478 and CFT-17826 from The Primary Screen

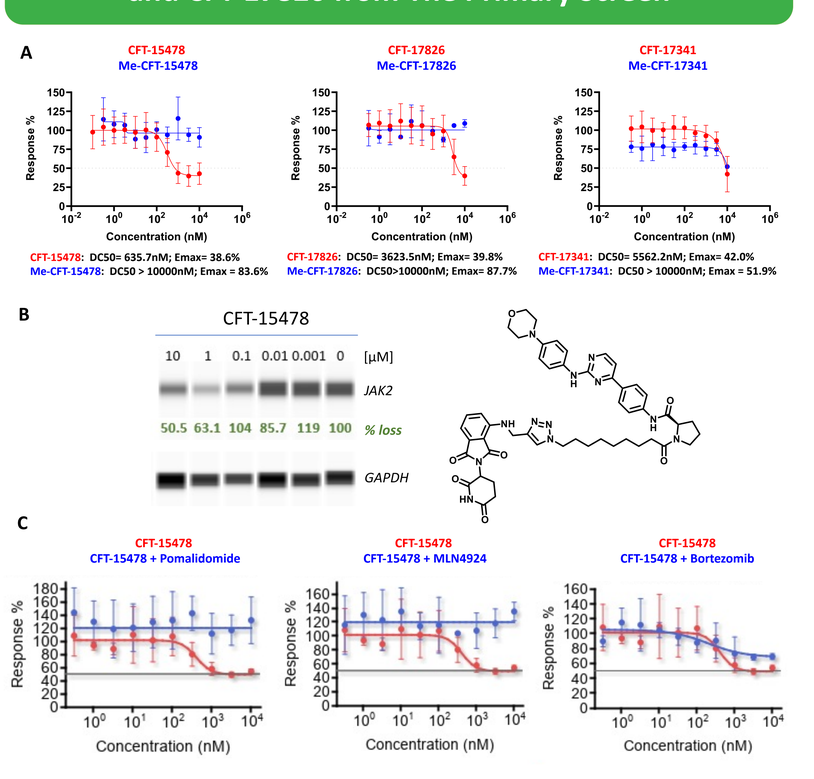


Figure 3: A, C HiBiT-detection of JAK2 with increasing concentration of JAK2 BiDAC degraders using HEL9217 cells with endogenously tagged JAK2 [V617F]. Bars represent SD. **A.** Red line represents JAK2 BiDAC degrader and blue line represent its matched CRBN-null degrader. **B** JAK2 level measured by western blot in HEL9217 cell line after 24hr of treatment with CFT-15478. GAPDH was used as a loading control. **C.** Red line represents CFT-15478 alone and blue line represents CFT-15478 in combination with Pomalidomide, MLN4924, or Bortezomib. CFT-15478 was dosed in 10 pts, semi-log, dose response while Pomalidomide, MLN4924, or Bortezomib was co-dosed in fixed concentration. The cells were treated for 24hr.

Co-dosing with JAK2 Ligand Did Not Rescue, But Enhanced, JAK2 Degradation Through PGP Inhibition

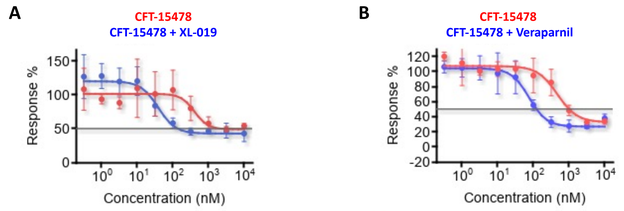


Figure 4: A, B HiBiT-detection of JAK2 with increasing concentration of JAK2 BiDAC degrader using HEL9217 cells with endogenously tagged JAK2 [V617F]. Bars represent SD. **A.** Red line represents CFT-15478 alone and blue line represents CFT-15478 in combination with XL-019. **B.** Red line represents CFT-15478 alone and blue line represents CFT-15478 in combination with Verapamil. CFT-15478 was dosed in 10 pts, semi-log, dose response while XL-019 or Verapamil was co-dosed in fixed concentration. The cells were treated for 24hr

Counter Screen Identified the Putative Hits as Potent GSPT1 Degraders, Accounting for the Observed JAK2 Loss

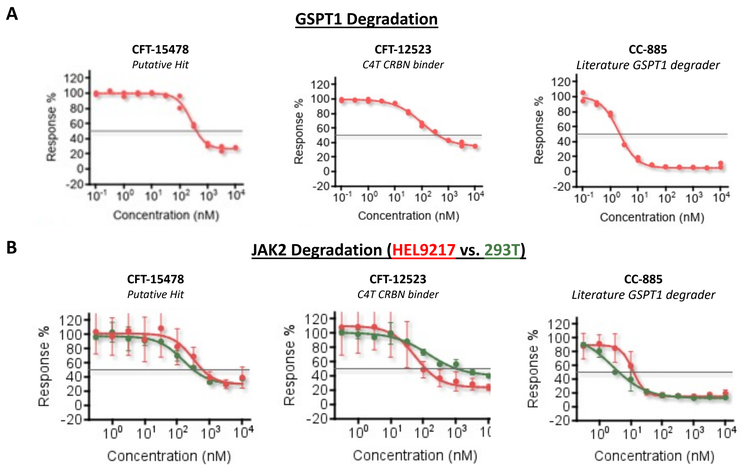


Figure 5: A. HiBiT-detection of GSPT1 with increasing concentration of CFT-15478, CFT-12523, or CC-885 using 293T with endogenously tagged GSPT1. Bars represent SD. **B.** HiBiT-detection of JAK2 with increasing concentration of CFT-15478, CFT-12523, or CC-885 using HEL9217 with endogenously tagged JAK2 [V617F] or 293T cells with overexpressed tagged JAK2 [V617F]. Red line represents HEL9217 cells and green line represents 293T cells.

Summary

- Three putative JAK2 BiDAC degraders were identified from a matrix library of 500+ BiDAC degraders targeting JAK2
- Comparison with matching CRBN binding-impaired degrader confirmed CRBN-dependent activity for CFT-15478 and CFT-17826
- Rescue studies with Pomalidomide/MLN 4924/Bortezomib demonstrated UPS-dependent mechanism for the CRBN-dependent putative hits
- Saturating levels of JAK2 ligand enhanced, instead of rescued, JAK2 degradation, which was attributed to PGP inhibition
- The cereblon-dependent hits were identified as degraders of GSPT1, a previously reported neo-substrate of cereblon
- This work demonstrates the importance of including GSPT1 counter screens and evaluation of PGP substrate potential in addition to on-mechanism degradation profiling approaches to ensure bona fide degrader hit confirmation

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