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EPriL: a platform for the rapid identification of degraders of clinically relevant BTK mutants

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Introduction

- We have developed our proprietary EPriL platform for the rapid identification of potent kinase degraders.
- Energetically Privileged Ligands (EPriLs), are based on a macrocycle that binds tightly in the ATP binding site and which has several exit vectors suitable for attachment of E3 ligase binders (see also abstracts 5814 and 1960).
- BTK inhibitor drugs are used in first line treatment for CLL, WM and as second line treatment for MCL [1].
- BTK degraders can combat inhibitor resistance by removing the scaffold function of BTK [2]. However, degraders in clinical trials such as NX-2127 and NX-5948 lack exhaustive activity on all resistance mutants.
- Here we apply our EPriL degrader platform to design pharmacologically novel BTK degraders with potent activity on all clinically relevant BTK inhibitor resistance mutants.

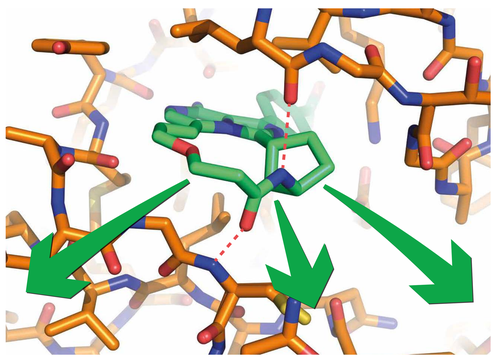

Figure 1: *X-ray structure of EPriL macrocycle binding in a kinase active site. Arrows indicate exit vectors suitable for attachment of E3 ligase binders. Red dashes indicate β-sheet complementation, a unique element of EPriL binding.*

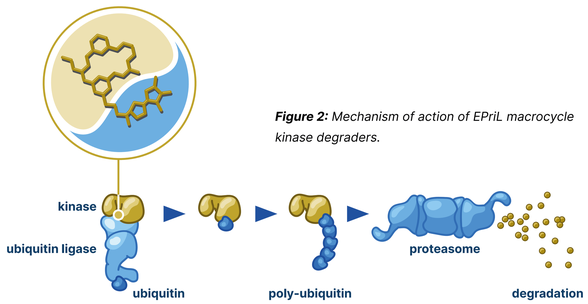

Figure 2: *Mechanism of action of EPriL macrocycle kinase degraders.*

Results

EPriL degrader library

High Throughput cellular degradation (HEK293 BTK HiBiT)

▼ *54 hits (DC_{50} < 1000 nM)*

Confirmation of degradation on Western Blot (TMD8, 100 nM)

▼ *8 hits*

Proliferation assay TMD8: BTK WT, BTK C481S, BTK T474I, BTK L528W, BTK V416L

Potency of degradation Western Blot (TMD8, dose range)

▼ *Chemical optimization of EPriL degraders*

HiBiT degradation assay, proliferation assays and Western Blots

▼ *Leads (DC_{50} < 100 nM)*

Second generation of optimized EPriL degraders

Figure 3: *Flow chart for identifying macrocycle-based BTK degraders that target BTK resistance mutations.*

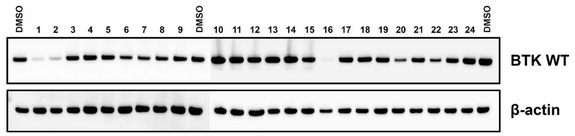

Figure 4: *BTK expression levels in TMD8 cells after 24 hours incubation with candidate BTK degraders (100 nM).*

First generation: CFON-466

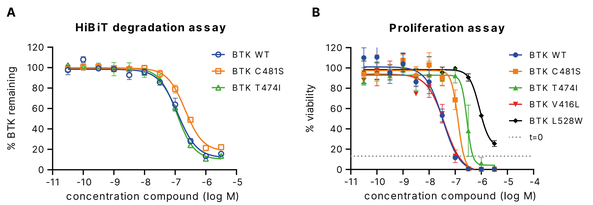

Figure 5: Effects of first generation EPriL BTK degrader CFON-466
(***A***) *BTK degradation in HEK293 cells overexpressing BTK-HiBiT constructs (24 h).* (***B***) *Antiproliferative effect on TMD8-derived cell lines with common BTK inhibitor resistance mutations (72 h).* (***C***) *Degradation of BTK in TMD8 BTK wild-type (WT) cells (24 h).*

Second generation: CFON-547

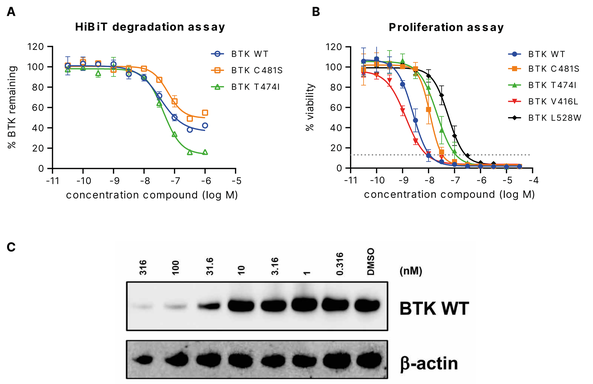

Figure 6: Effects of second generation EPriL BTK degrader CFON-547
(***A***) *BTK degradation in HEK293 cells overexpressing BTK-HiBiT constructs (24 h).* (***B***) *Antiproliferative effect on TMD8-derived cell lines with common BTK inhibitor resistancemutations (72 h).* (***C***) *Degradation of BTK in TMD8 BTK WT cells (24 h).*

TMD8 BTK variant (IC_{50}, nM)

	WT	C481S	T474I	V416L	L528W
CFON-601	4.6	23	37	3.5	67
CFON-547	2.1	14	24	1.3	52
CFON-682	3.0	17	34	1.8	66
NX-2127	7.9	99	58	48	212
NX-5948	1.1	18	5.1	18	159

Figure 7: *Antiproliferative potency of lead EPriL BTK degraders on TMD8 cell lines with the most common BTK inhibitor resistance mutations (72h), and comparison with clinical stage BTK degraders NX-2127 and NX-5948.*

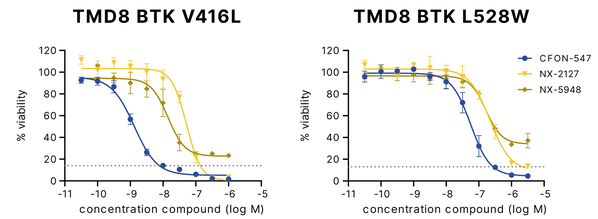

Figure 8: *Antiproliferative effect of EPriL BTK degrader CFON-547 on TMD8 BTK V416L and TMD8 BTK L528W cell lines, compared to clinical stage BTK degraders NX-2127 and NX-5948.*

Conclusion

- Our EPriL macrocycle platform allows for the rapid identification of potent kinase degraders.
- EPriL-based lead BTK degraders efficiently induce proteolytic removal of BTK WT and resistance mutants BTK C481S, BTK T474I, BTK L528W, and BTK V416L, leading to potent antiproliferative effects in BTK-dependent cell lines.
- Our lead BTK degraders such as CFON-547 have better antiproliferative potency and efficacy than NX-2127 and NX-5948 in TMD8 lines harboring BTK resistance mutants such as BTK V416L and BTK L528W.

References: [**1**] Wang *et al.*, N Engl J Med 2022; 386:735-743, [**2**] Montoya *et al.*, Science 383, eadi5798 (2024).