Abstract # LB-010

Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4

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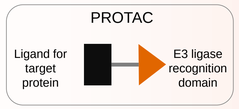
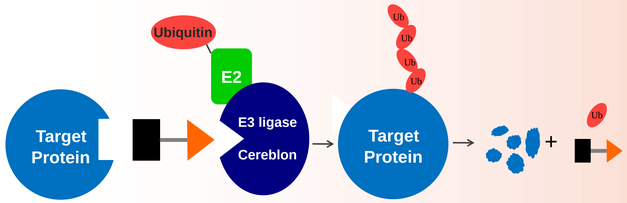
Abstract:

- BRD4, a member of the bromodomain and extraterminal domain (BET) family, has emerged as an attractive oncology target.
- BET bromodomain inhibitors have showed promising effects in certain preclinical settings, particularly in c-MYC driven hematological malignancies.
- Interestingly, we found that BRD4 inhibitors lead to a rapid and robust accumulation of BRD4, which may partially account for their moderate suppression of c-MYC and inhibition of cell proliferation.
- We designed a Proteolysis Targeting Chimera (PROTAC) compound, containing a BRD4 binding moiety and an E3 ubiquitin ligase cereblon ligand.
- BRD4 PROTAC leads to fast and efficient degradation of BRD4.
- BRD4 PROTAC provides more pronounced and longer-lasting effect in suppressing c-MYC levels than small molecule BRD4 inhibitors.
- BRD4 PROTAC is more effective in inhibiting Burkitt Lymphoma cell proliferation and inducing apoptosis compared to BRD4 inhibitors.
- A cereblon-based PROTAC provides a better and more efficient strategy in targeting BRD4 than traditional small molecule inhibitors.

Degrader Technology

PROTAC: PROteolysis TArgeting Chimera

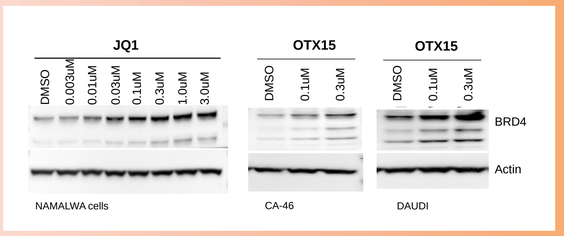




E3 Ligase is recruited to the target protein by PROTAC

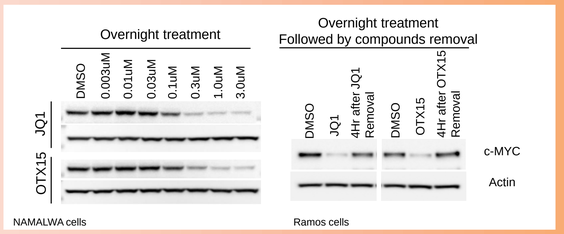
Tagged target is recognized and degraded by proteasome machinery

BRD4 inhibitors lead to fast and robust BRD4 accumulation

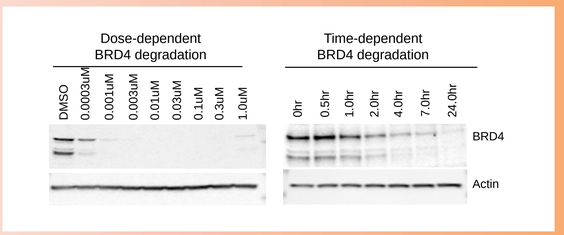


Various BL cell lines treated with small molecule inhibitors overnight

BRD4 inhibitors lead to inefficient and transient suppression on c-MYC

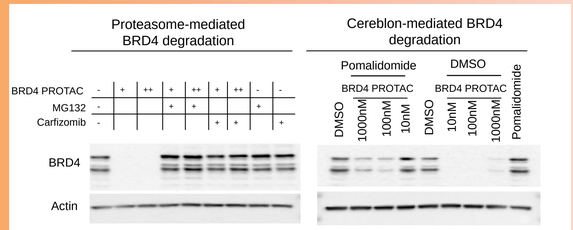


BRD4 PROTAC causes rapid and potent BRD4 degradation



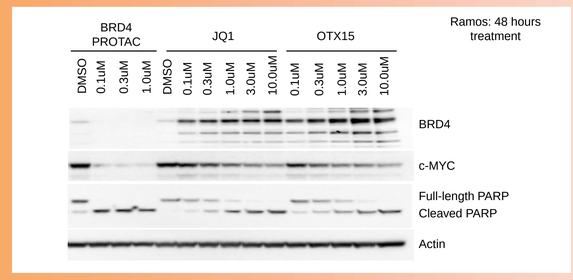
NAMALWA cells

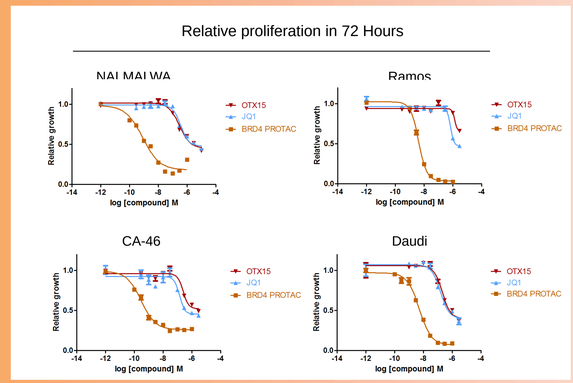
Confirmation of proteasome mediated, cereblon-based mechanism in driving BRD4 degradation by BRD4 PROTAC



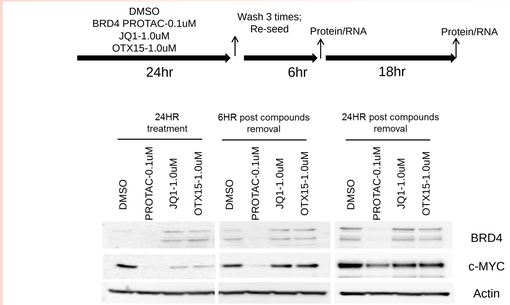
NAMALWA cells, + (10nM), ++ (100nM), MG132 (5uM), Carfizomib (5uM), Pomalidomide (10uM)

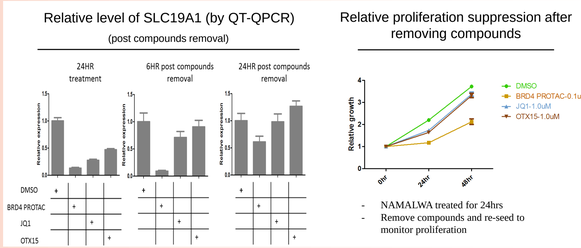
BRD4 PROTAC leads to more significant c-MYC suppression, proliferation inhibition and apoptosis induction than small molecule inhibitors





BRD4 PROTAC provides sustained effects on BRD4 degradation, c-MYC and its downstream signal suppression and proliferation inhibition





- NAMALWA treated for 24hrs
- Remove compounds and re-seed to monitor proliferation

Summary

- We successfully developed a BRD4 PROTAC that degrades BRD4 potently and rapidly.
- BRD4 PROTAC causes more significant suppression of c-MYC, more dramatic effect on proliferation inhibition and apoptosis induction compared to small molecule inhibitors.
- BRD4 PROTAC has long-lasting effect on repressing downstream signaling and proliferation.

❖ Targeted degradation of BRD4 by PROTAC provides a better and more effective strategy in targeting BRD4 than traditional small molecule inhibitors.

❖ Our study also demonstrates that PROTAC platform, by actively recruiting E3 ligase to target pathological protein for degradation, is a promising strategy in targeting the “undruggable” proteins by traditional approaches.