In vitro assays to de-risk new cancer therapeutics: comparing the effects of BH3-mimetics on platelet viability

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Background

The anti-apoptotic BCL-2 proteins have been investigated as targets in cancer therapy. While ABT-199 (Venetoclax) has been an early success, new therapeutics including ABT-737 and ABT-263 (Navitoclax) have been limited by on-target and dose-limiting thrombocytopenia (diagram 1)[1,2].

The PROTAC (Proteolysis Targeting Chimera) DT2216 was designed to avoid the on-target platelet toxicity of ABT-263 (diagram 2). Initial *in vitro* data reported DT2216 to be platelet sparing,[3,4] but later platelet toxicity was reported in mouse, rat and cynomolgus monkey models[5].

More sensitive and reliable *in vitro* platelet viability assays are required to better predict *in vivo* toxicity in early drug discovery and development.

Conclusion

Using BH3-mimetics with known effects on platelet viability (ABT-199, ABT-263, ABT-737), we have demonstrated the suitability of sensitive, higher throughput *in vitro* platelet assays for predicting *in vivo* platelet toxicity.

Furthermore, in contrast with *in vitro* data in the literature, DT2216 was found to decrease platelet viability and increase caspase 3/7 activity in our *in vitro* platelet toxicity tests. These findings better mirror the early results of *in vivo* studies in animal models.

Improved *in vitro* assays with the capability for screening BH3-mimetics and other therapeutics in development are available to help de-risk the selection and progression of new drug candidates in drug development.

Modes of Action

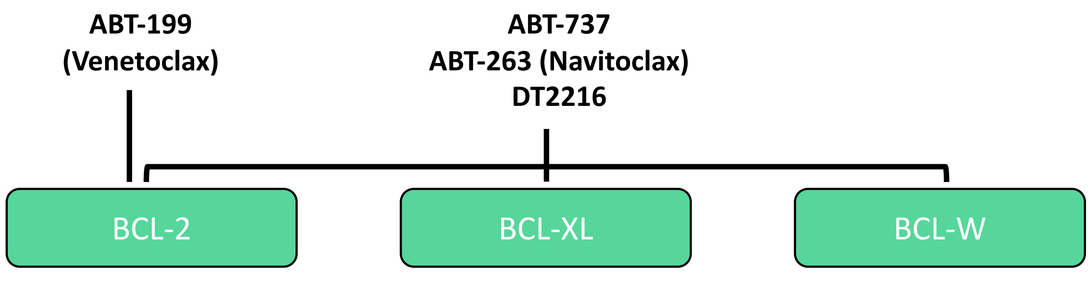

Diagram 1. Summary of the BH3-mimetics and DT2216 and their anti-apoptotic protein targets
The more specific ABT-199 targets BCL-2 only while ABT-737, ABT-263 and DT2216 target BCL-2, BCL-XL and BCL-W.

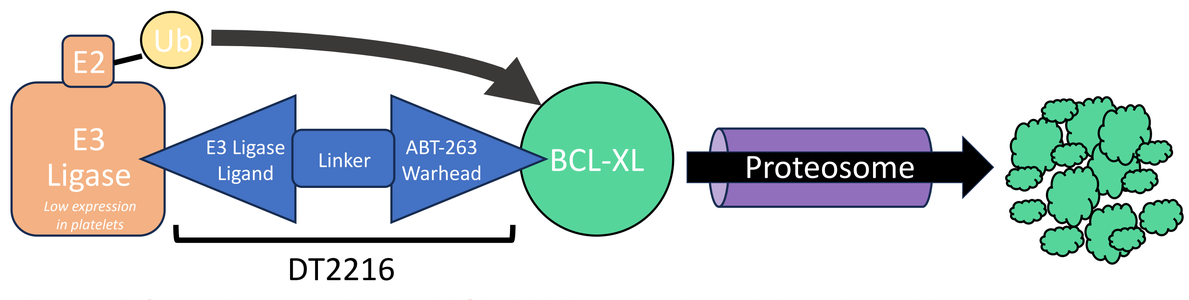

Diagram 2. Schematic representing the PROTAC DT2216 interaction with the anti-apoptotic protein target BCL-XL
DT2216 is comprised of a modified ABT-263 warhead, that binds anti-apoptotic proteins, and an E3 ligase ligand. Recruitment of E3 ligase to the target protein leads to the transfer of ubiquitin (Ub) and degradation of the target protein.

Results

Effects of the compounds ABT-199, ABT-263, ABT-737, and DT2216 were assessed in human washed platelets at 37°C, initially using the MTS assay. A subsequent study was performed with cell titre blue and caspase 3/7 activity assays, as these can be multiplexed.

- Although ABT-199 had little to no effect on platelet viability after 3 h, slightly elevated levels of caspase 3/7 activity were observed. A reduction in platelet viability was measured following 24 h treatment and this was corroborated by increased levels of caspase 3/7 activity.
- The pan-BCL inhibitors ABT-737 and ABT-263 both reduced platelet viability and increased caspase 3/7 activity after 3 h treatment and their potency increased at 24 h.
- DT2216 was shown to reduce platelet viability and increase caspase 3/7 activity following 24 h incubation.
- The order of potency in cell titre blue and caspase 3/7 activity following 24 h incubation was: ABT-263 > DT2216 = ABT-737 > ABT-199

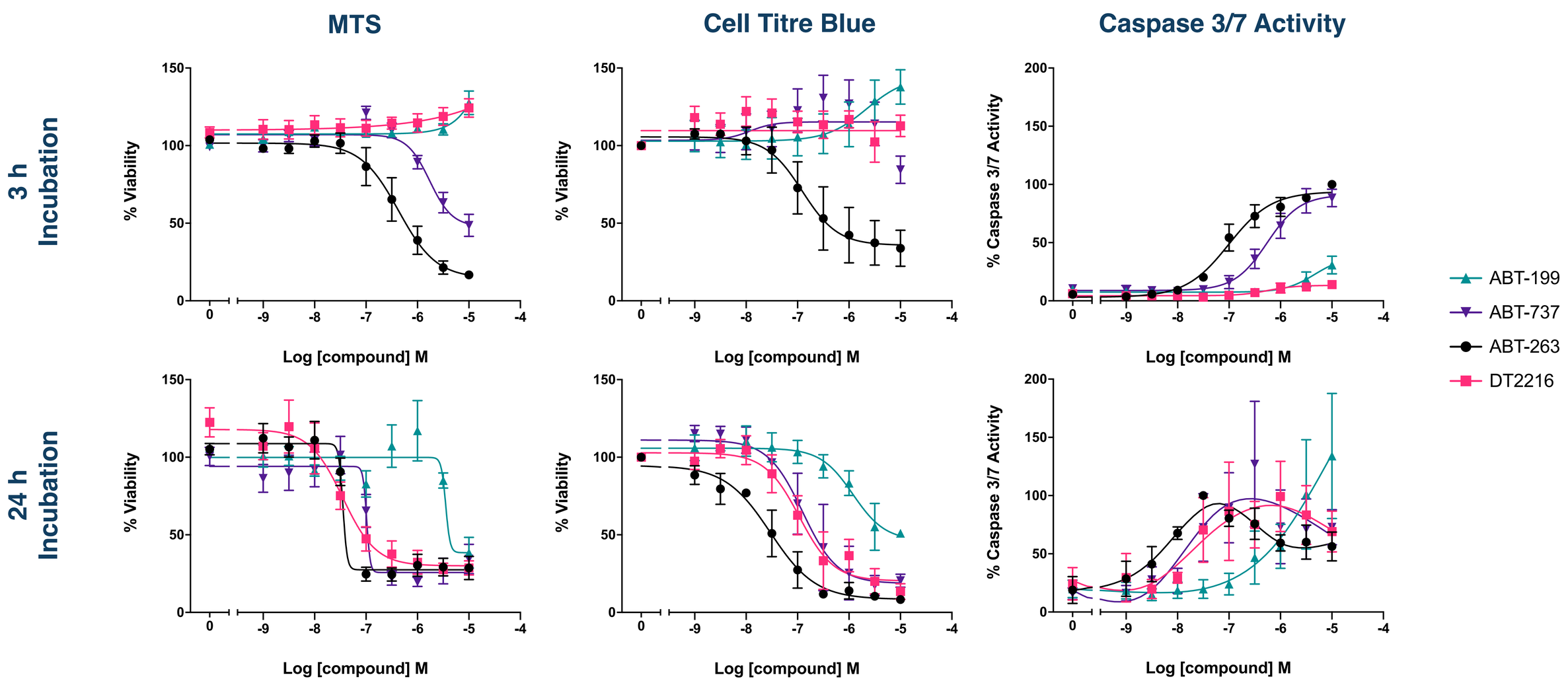

Figure 1. The effects of the BH-3 mimetics ABT-199, ABT-737, ABT-263 and PROTAC DT2216 on human platelets
The effects of ABT-199, ABT-263, ABT-737 and DT2216 were assessed following 3 h and 24 h incubation with washed platelets in three different assays: MTS (n=5-6), cell titre blue (n=3-4), and caspase 3/7 activity (n=3-4). All data are mean ± SEM.

References: (1) Bcl-x_L–inhibitory BH3 mimetics (ABT-737 or ABT-263) and the modulation of cytosolic calcium flux and platelet function (S.M. Schoenwaelder & S.P. Jackson 2012). (2) BH3-mimetics: recent developments in cancer therapy (P.A. Townsend et al., 2021), (3) A selective BCL-X_L PROTAC degrader achieves safe and potent antitumor activity (S. Khan et al., 2019), (4) DT2216-a Bcl-xL-specific degrader is highly active against Bcl-xL-dependent T cell lymphomas (Y. He et al., 2020). (5) Discovery of BCL-XL heterobifunctional degrader with potentially improved therapeutic window and minimal platelet toxicity for hematological malignancies (Y. Xie et al., 2023).