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ORAL ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Discovery of BCL-XL Heterobifunctional Degrader with Potentially Improved Therapeutic Window and Minimal Platelet Toxicity for Hematological Malignancies

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Background
In hematologic malignancies, apoptosis evasion promotes tumor formation/progression and is driven by the overexpression of anti-apoptotic proteins, including BCL-2 and BCL-XL. BCL-2-targeting small molecule inhibitor venetoclax won FDA approval for chronic lymphocytic leukemia and acute myeloid leukemia. Interests in developing BCL-XL-selective inhibitors arise as BCL-XL overexpression is implicated in venetoclax resistance. However, these efforts failed in clinical development due to dose-limiting thrombocytopenia, as platelets (PLT) also depend on BCL-XL for survival. To mitigate this on-target toxicity, a degrader strategy has been proposed, whereby BCL-XL protein can be selectively degraded by the von Hippel-Lindau protein (VHL) E3 ligase in tumor cells, but not in PLT which minimally express VHL. The first such BCL-XL degrader (DT2216) was developed and is now in early clinical trials. Despite negligible PLT toxicity *in vitro*, DT2216 was still toxic to PLT *in vivo*.
We reasoned that since earlier BCL-XL degraders used strong BCL-XL inhibitor as warhead, *e.g.* the warhead for DT2216 is ABT263, they could readily bind to and inhibit BCL-XL in PLT causing thrombocytopenia. Warhead-containing metabolites could also form and behave as an inhibitor, exacerbating PLT toxicity *in vivo*. Utilizing our artificial intelligence-empowered neoDegrader platform, which accurately characterizes the landscape of the degrader-mediated, metastable interactions between E3 and its target protein, we were able to computationally design molecules that were relatively weak BCL-XL binders but could maintain the ternary complex at metastable states. Herein, we report the preclinical profile of the lead candidate NXD02.
Methods
We assessed the binding affinity of NXD02 to BCL-XL by surface plasmon resonance. We conducted flow cytometry to quantify NXD02-mediated BCL-XL protein degradation and apoptotic effects in MOLT4 cells. We also quantified the anti-proliferative activity of NXD02 by CellTiter-Glo assay. We measured protein level changes of apoptosis markers by western blot. We simultaneously determined plasma concentration of NXD02 and PLT at various time points following a single injection of NXD02 in rodents and monkeys. Finally, we evaluated the anti-tumor activity of NXD02 in an orthotopicMOLT4-luc mouse xenograft (CDX) model by bioluminescence imaging.
Results
The binding affinity of NXD02 to BCL-XL was > 40-fold weaker than that to DT2216 (K_D 28.7 vs 0.6 nM). Despite this, NXD02 strongly degraded BCL-XL protein (DC_{50} 6.6 nM) with no obvious hook effect, which was more potent than DT2216 (DC_{50} 17.4 nM) in MOLT4 cells. At 100 nM, NXD02 markedly increased levels of active caspase 3 and cleaved PARP, which was consistent with evidence of massive apoptosis. This was translated to a potent anti-proliferative effect comparable to DT2216 (IC_{50} 27.8 vs 42.7 nM).
NXD02 did not affect PLT viability up to a concentration of 10 μM *in vitro*. Notably, a single dose of NXD02 at 10 mg/kg *i.v.* achieved an exposure >4-fold than that of DT2216 (dose-normalized AUC 68.5 vs 15.3 μg*h/mL/(mg/kg)) in CD1 mice, while producing similar levels of peak PLT reduction (58% vs 65%) at ~24 h, which recovered by day 3. Comparable or less PLT reduction was also observed in rats (64% vs 66%) at 15 mg/kg and in cynomolgus monkeys (63% for NXD02 vs 87% for DT2216) at 3 mg/kg, all of which fully recovered by days 5-6, supporting the possibility for weekly dosing.
Furthermore, NXD02 demonstrated stronger *in vivo* anti-tumor activity than DT2216 in a CDX model. Weekly i.v. dosing of NXD02 at 3 and 10 mg/kg achieved 80% and 100% tumor growth inhibition (TGI) by day 28 respectively, as compared to 36% TGI for DT2216 at 10 mg/kg. There was no significant body weight loss for all groups.

Conclusions
We have discovered a structurally-novel, VHL-mediated, heterobifunctional BCL-XL degrader NXD02 with "Weak Inhibitor, Strong Degrader" properties. NXD02 displayed, at the same dose, a much higher drug exposure, better anti-tumor activity and comparable platelet toxicity profile *in vivo* than the most advanced BCL-XL degrader DT2216. The results suggest that NXD02 may have a more favorable safety margin. *In vitro* toxicity profiling of NXD02 including Ames test and safety panel revealed no concerns so far. *In vivo* safety assessment is ongoing for NXD02 as a promising candidate for clinical development in liquid and potentially select solid tumors.

Disclosures Xie: *neoX Biotech:* Current Employment, Current equity holder in private company. **Xing:** *neoX Biotech:* Current Employment. **Lai:** *neoX Biotech:* Current Employment. **Tao:** *neoX Biotech:* Current Employment. **Gu:** *neoX Biotech:* Current Employment. **Cao:** *neoX Biotech:* Current Employment. **Diao:** *neoX Biotech:* Current Employment. **Bai:** *neoX Biotech:* Current Employment. **Chen:** *neoX Biotech:* Current Employment, Current equity holder in private company. **Lei:** *neoX Biotech:* Current Employment. **Guo:** *neoX Biotech:* Current Employment, Current equity holder in private company; *I-Mab Biopharma:* Current equity holder in publicly-traded company, Ended employment in the past 24 months.

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