ELSEVIER

Blood 142 (2023) 4158–4159

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Novel Hypomethylating Agent Ntx-301 Exhibits Anti-Leukemia Activity in Venetoclax-Resistant and TP53-Mutant AML

Bing Z. Carter, PhD[1], *Po Yee Mak*[1], *Baozhen Ke*[1], *Andrea Bedoy*[1], *Steffen Boettcher, MD*[2], *Lauren B. Ostermann, BSc*[1], *Elizabeth J. Shpall, MD*[3], *Michael Andreeff, MD PhD*[1]

[1] Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX
[2] University Hospital Zurich, Zurich, Switzerland
[3] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX

The FDA approved Bcl-2 inhibitor venetoclax/hypomethylation agent (HMA) combination has greatly improved outcomes in patients with AML. However, despite of high response rates, the median overall survival is only 14.7 months and 2.5 months post relapse. Novel therapies in therapy-resistant AML are urgently needed. Multiple mechanisms contribute to resistance to venetoclax therapies including mutations of oncogenic kinases, leading to constitutive activation of the FLT3, SHP2, and RAS signaling cascade and upregulation of antiapoptotic Bcl-2 proteins, and mutations of TP53. We here investigate the therapeutic efficacy of NTX-301 (5-aza-thio 2'-deoxycytidine) (PinotBio, Inc.), a novel HMA in venetoclax-resistant and TP53-mutant AML.

NTX-301 greatly reduced the levels of DNMT1 and decreased viability in various AML cells, markedly more effective than 5-azacytide (5-Aza). NTX-301 decreased Mcl-1 and effectively induced cell death in AML cells overexpressing Mcl-1 and Bcl-2A1, both of which are upregulated by various kinases and resistant factors to venetoclax; in AML cells with intrinsic or acquired resistance to venetoclax; and in AML cells and stem/progenitor cells obtained from patients who were resistant/relapsed from venetoclax-based therapies. Furthermore, cell death induction in aforementioned cells was synergistically enhanced when NTX-301 was combined with venetoclax. NTX-301 alone or venetoclax/NTX-301 combination had minimal activity in normal bone marrow cells and bone marrow $CD34^+$ stem/progenitor cells. Importantly, using a PDX model developed from an AML patient who relapsed from venetoclax/HMA, NTX-301 alone demonstrated great anti-leukemia activity and significantly extended survival (167 vs 114 days of control, P=0.0001), while venetoclax or venetoclax/5-Aza did not. CyTOF analysis of mouse bone marrow cells collected at the end of 5 week treatment showed that NTX-301 and even more so NTX-301/venetoclax effectively decreased $CD45^+$ leukemia blast cells and $CD34^+CD38^+$/$CD34^+CD38^-$ AML stem/progenitor cells, while venetoclax or venetoclax/5-Aza had not effects.

We observed that NTX-301 activated p53 in TP53 wild-type AML cells. We also observed that NTX-301 increased p73 and decreased MDM2 in TP53-mutant cells and showed activities in TP53-mutant leukemia cells. NTX-301 and venetoclax combination synergistically induced cell death and decreased viable cells in AML cells and stem/progenitor cells with TP53 mutations. Additionally, NTX-301 treatment increased caspase-8 and cleaved-caspase-8 in AML cells independent of TP53 mutation status, consistence with reports showing caspase-8 hypermethylation in AML and supporting that activation of caspase-8-mediated extrinsic apoptosis as a mechanism of NTX-301 action.

In conclusion, our data suggest that NTX-301 has more potent anti-leukemia activities compared to current HMA in clinic and synergizes with venetoclax in venetoclax-resistance and TP53-mutant AML and AML stem/progenitor cells, and warrants clinical evaluation

Disclosures Carter: *Syndax:* Research Funding; *PMV:* Research Funding; *Revolution Medicines:* Research Funding; *PinotBio:* Research Funding. **Shpall:** *Syena:* Other: License agreement; *Navan:* Membership on an entity's Board of Directors or advisory committees; *Adaptimmune:* Membership on an entity's Board of Directors or advisory committees; *Axio:* Membership on an entity's Board of Directors or advisory committees; *Takeda:* Other: License agreement; *Affimed:* Other: License agreement; *Celaid Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *NY Blood Center:* Membership

on an entity's Board of Directors or advisory committees; *Fibrobiologics:* Membership on an entity's Board of Directors or advisory committees. **Andreeff:** *PMV:* Research Funding; *Kintor Pharmaceutical:* Research Funding.

https://doi.org/10.1182/blood-2023-180051