# A STUDY ON THE PRECLINICAL EFFICACY, UNDERLYING MECHANISMS, AND SENSITIVITY MARKERS OF A NOVEL HYPOMETHYLATING AGENT NTX-301 IN ACUTE MYELOID LEUKEMIA


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## INTRODUCTION

- Elderly patients with acute myeloid leukemia (AML) are ineligible for standard intensive chemotherapy. Thus, their clinical management remains challenging.
- As alternative strategies, less-intensive regimens with hypomethylating agents (HMAs) alone or in combination with the BCL-2 inhibitor venetoclax (VCX) are currently being used in these unfit patients.
- Due to low response rates and adverse events of conventional HMAs, novel HMAs that elicit enhanced efficacy and reduce mortality rates are required.
- A novel HMA, 5-aza-4'-thio-2'-deoxycytidine (NTX-301), showed a promising preliminary result for the safety and efficacy.

## AIM

- The antileukemic activity, mechanisms of action (MoAs), and predictive biomarkers of NTX-301 are not yet understood.
- We aim to thoroughly investigate the experimental and preclinical efficacy of NTX-301 through comparative analyses with conventional agents decitabine (DAC) and azacitidine (AZA), which have been well defined as therapeutics for AML.

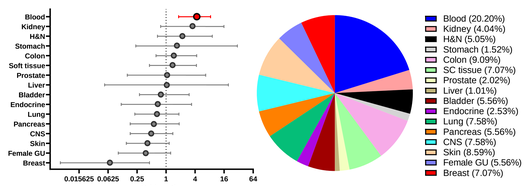
## METHOD

- To comprehensively evaluate the anticancer activity of NTX-301, we examined the viability of 200 cancer cell lines (CCLs) upon NTX-301 treatment based on two sensitivity metrics: the $IC_{50}$ and the area under the dose-response curve (AUC).
- To assess the antileukemic activity of NTX-301, we performed in vitro cell-based phenotypic assays, including assays to analyze cell viability, cell cycle, apoptosis, and AML cell differentiation.
- We evaluated the preclinical efficacy of NTX-301 as monotherapy and in combination with VCX by establishing six different mouse models encompassing both systemic and subcutaneous xenografts.
- To identify the MoAs underlying the antileukemic activity and the predictive biomarkers of NTX-301, we analyzed multiomics data.

## RESULTS

### ❖ *Sensitivity profiling of NTX-301 across 199 cancer cell lines (CCLs)*

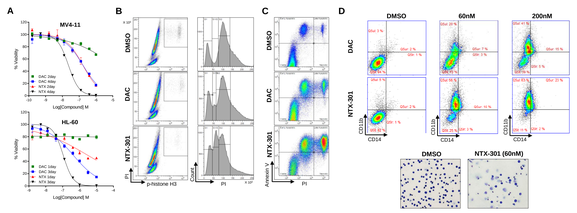
- Consistent with the current use of HMAs as therapeutics for hematologic malignancies, NTX-301 displayed skewed sensitivity toward CCLs of hematopoietic origin.
- Compared with the median AUC of hematopoietic CCLs, NTX-301 achieved higher efficacy in 21.3% of solid CCLs, whereas DAC achieved higher efficacy in only 0.89% of solid CCLs.
- Thus, sensitivity profiling revealed that NTX-301 not only primarily sensitizes hematologic malignancies but also has better efficacy than conventional HMAs against solid cancers.




### ❖ *Comparative analysis reveals the superior antileukemic activity of NTX-301*

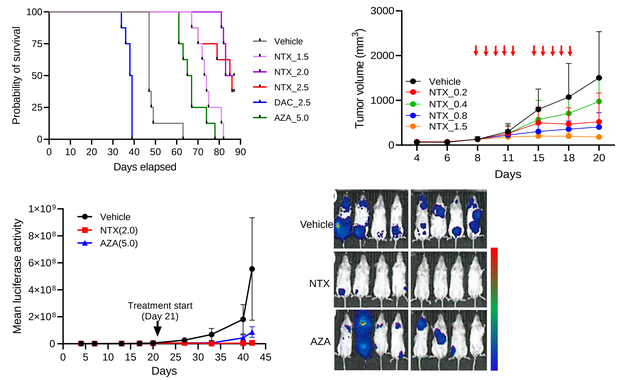
### ❖ In vitro cell-based assays

- The superior antileukemic activity of NTX-301 was attributed to its more effective induction of cytotoxicity (A), cell cycle arrest(B), apoptosis (C), and differentiation (D).



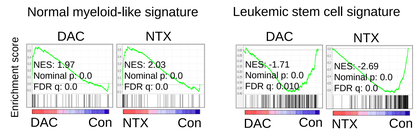

### ❖ In vivo mouse studies

- Preclinical studies demonstrated the improved antileukemic activity, tolerability, and survival outcomes of NTX-301.

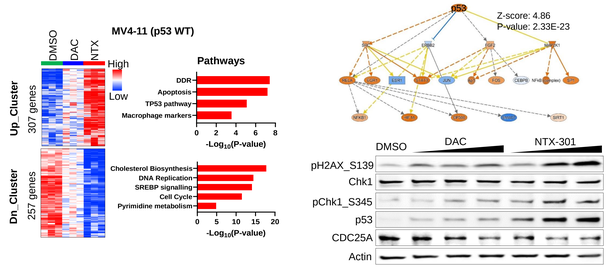



### ❖ *Transcriptome analysis reveals the antileukemic MoAs of NTX-301*

- NTX-301 promoted greater transcriptional reprogramming toward a normal myeloid-like signature, accompanied by stronger suppression of the leukemic stem cell signature.

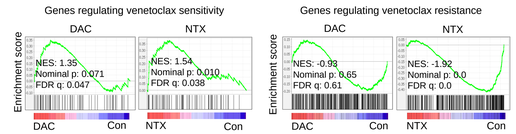



- NTX-301 activated biological processes such as the DNA damage response (DDR), the p53 pathway, the immune response, and apoptosis.

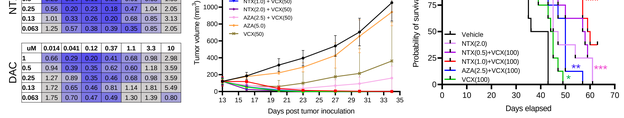



### ❖ *Clinical benefits of the antileukemic MoAs of NTX-301 in combination with the BCL-2 inhibitor venetoclax*

- NTX-301 triggered more marked transcriptional reprogramming toward sensitivity to VCX than did DAC; it significantly up- and downregulated genes driving sensitivity and resistance, respectively, thus priming AML cells for higher sensitivity.

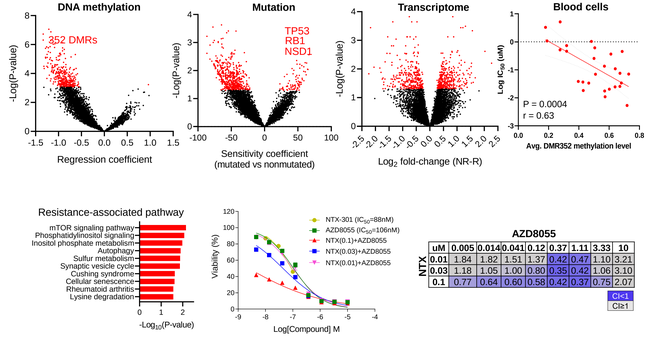



- The combination of NTX-301+VCX resulted in a combination index (CI) implying greater synergy than DAC+VCX and achieved complete tumor remission and prolonged survival.




### ❖ *Predictive biomarkers associated with sensitivity/resistance to NTX-301*

- Integrative analysis of multiomics data and sensitivity profiles in 199 CCLs revealed a methylome-based marker DMR352, mutations frequently occurring in nonresponders in three genes, *TP53*, *RB1*, and *NSD1,* and mTOR-driven intrinsic resistance.




AZD8055

| NTX | uM | 0.005 | 0.014 | 0.041 | 0.12 | 0.37 | 1.1 | 3.33 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| | 0.01 | 1.84 | 1.82 | 1.51 | 1.37 | 0.42 | 0.47 | 1.10 | 3.21 |
| | 0.03 | 1.18 | 1.05 | 1.00 | 0.80 | 0.35 | 0.42 | 1.06 | 3.10 |
| | 0.1 | 0.77 | 0.64 | 0.60 | 0.58 | 0.42 | 0.37 | 0.75 | 2.07 |

CI<1
CI≥1

## CONCLUSION

- These findings highlight the improved therapeutic index of NTX-301 and the underlying mechanisms and predictive biomarkers, providing a rationale for its clinical development.
- Our study will enhance our understanding of NTX-301 and contribute to patient stratification during clinical development, thus guiding a novel therapeutic option for elderly patients with AML.