ANTI-ADHESION PROPERTIES OF KTX-1001, A SELECTIVE NSD2/MMSET INHIBITOR, ENHANCE CARFILZOMIB SENSITIVITY IN MULTIPLE MYELOMA

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BACKGROUND

In t(4;14) multiple myeloma (MM), the histone methyltransferase **NSD2** is dysregulated through juxtaposition with the IgH super-enhancer, resulting in overexpression and aberrantly high levels of **H3K36 dimethylation (H3K36me2)**. Elevated NSD2 activity promotes MM cell growth, proliferation, and enhanced cell–cell and cell–matrix adhesion within the bone marrow (BM) microenvironment.

KTX-1001 is an oral, small-molecule NSD2 inhibitor currently under evaluation in a Phase 1 trial in late-stage MM (NCT05651932).[1]

Objective: We present the initial characterization of KTX-1001 in MM cell lines along with biomarker analyses from patient-derived BM samples.

Key findings: 1) KTX-1001 disrupts MM cell adhesion by modulating **CD44, CD56, and N-cadherin** expression. **2)** In a bortezomib-resistant, highly adherent MM cell line, KTX-1001 demonstrates **synergy with carfilzomib (CFZ)**.

AIMS

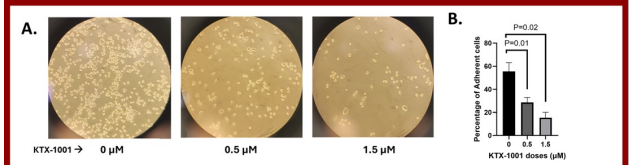
- Characterize the **mechanism of action** of KTX-1001 in multiple myeloma and **examine** the impact of KTX-1001 on MM cell adhesion.
- **Investigate the effect of adhesion disruption** on sensitivity to CFZ and **assess potential synergy** between KTX-1001 and CFZ in resistant, highly adherent MM cells.

METHODS

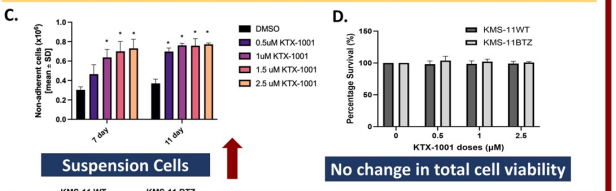
KMS11 wildtype (WT) and bortezomib-resistant (BTZ) cells were treated with escalating doses of **KTX-1001** to assess proliferation and adhesion. Suspension cell changes were evaluated by Matrigel assays (days 7, 11). **CellTiter-Glo** measured viability and synergy with **carfilzomib**. Adherent vs. suspension fractions of KMS11 WT cells were tested in CFZ dose–response assays. **Colony formation assays** assessed effects on cell–cell interaction. **RNA-seq** and **Western blot** profiled gene and protein expression, and **patient bone marrow samples** were analyzed by mass cytometry.[2]

RESULTS

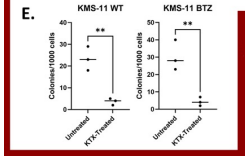
1. KTX-1001 Treatment Disrupts Cell Adhesion



Reduction in Adherent cell population with increasing doses of KTX-1001

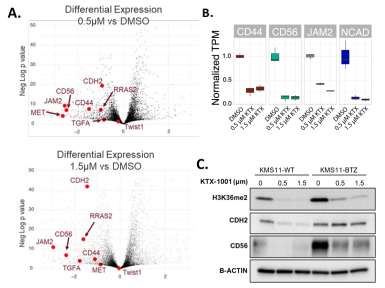


Suspension Cells — **No change in total cell viability**



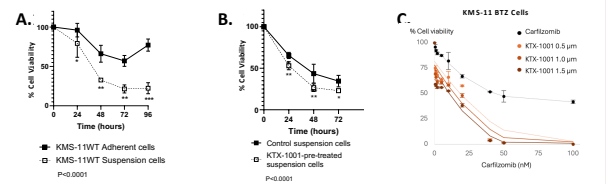
(A) And (B) **Dose-dependent reduction** in the number of adherent cells. (C) Dose- and time- dependent increase in suspension cells. (D) No significant difference in the overall cell viability. (E) Significant decrease in colony formation efficiency of KMS-11 wildtype and BTZ-resistant cells post KTX-1001 treatment.

2. Transcriptomic and Protein Analyses Reveal Downregulation of Adhesion Pathway Targets



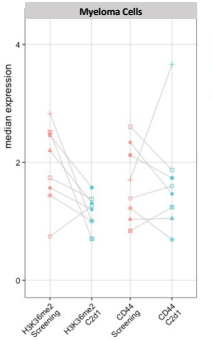
(A) And (B) **Dose-dependent downregulation of expression of adhesion pathway genes** following KTX-1001 treatment of KMS-11 wildtype cells. (C) Western blot showing downregulation of H3K36me2, CD56 (NCAM1) and CDH2 (N-CADHERIN) following KTX-1001 treatment for 7 days.

3. Anti-adhesive Properties of KTX-1001 Enhanced Sensitivity to Carfilzomib



(A) The adherent and suspension fraction of KMS-11 wildtype (semi-adherent) cells were separately tested for carfilzomib sensitivity using Cell-Titer Glo assay. **The suspension cells were significantly more sensitive to CFZ** compared to adherent cells. (B) KMS-11 wildtype adherent cells were treated with KTX-1001 (control adherent cells were DMSO-treated). After 72 hours, the 'control suspension cells' that were naturally released from DMSO-treated adherent cells as well as the KTX-1001-pre-treated suspension cells were tested for CFZ sensitivity. **The KTX-1001-pre-treated suspension cells were significantly more sensitive to CFZ** compared to the control suspension cells. (C) KTX-1001 shows **synergised cell killing with CFZ** on KMS-11 BTZ resistant cells

4. KTX-1001 Treatment Downregulated H3K36me2 and Adhesion Genes in Patient Bone Marrow



- Differential gene expression analysis demonstrated that **decreased H3K36me2 in MM cells was the most significant** (p=7.7e-03) on-treatment change observed at Cycle 2. As expected, a corresponding increase in K27me3 was also observed.
- CD44 and CD56 were decreased in MM cells in 5/8 patients.
- Analysis of TME cell subsets is ongoing.

Patient BMMCs from screening and Cycle 2 Day 1 (C2d1) were analysed by mass cytometry for H3K36me2, the KTX-1001 primary PD marker, as well as secondary PD markers, including adhesion genes.

CONCLUSIONS

- **NSD2 inhibition by KTX-1001 disrupted MM cell adhesion**, leading to:
  - Dose- and time-dependent increase in suspension cells.
  - Reduced colony formation in both parental and proteasome inhibitor (PI)–resistant cells.
- Disruption of adhesive properties of MM cells were mediated through modulation of adhesion markers (**CD56, CD44, N-cadherin**) in cell lines and patient bone marrow samples.
- **KTX-1001–induced adhesion disruption,** sensitized bortezomib (BTZ)-resistant cells to combination treatment with **carfilzomib (CFZ)**
- Ongoing trial analyses of **KTX-1001 + CFZ** patient samples will help validate synergy and confirm the proposed molecular mechanism in MM.

REFERENCES

1. Bories P, et al. *Blood.* 2024; 144(Suppl1):3370.
2. Lourenco J, et al. *Blood* 2024; 144(Suppl1):6814.

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