Anti-adhesion Properties of KTX-1001, a Selective NSD2/MMSET Inhibitor, Enhance Carfilzomib Sensitivity in Multiple Myeloma

Devi Nandana[1]*, Erin Flynt[2]*, Hui Choo[1], Seyed Alireza Hasheminasab[1], Warren Baker[1], Vicki Gamble[1], Mohammad Kazeroun[1], Vinidhra Sridharan[2], Nikhil Munshi[3,4], Mehmet Samur[5,6], Anjan Thakurta[1]

*Equal contribution.

Affiliations:

1. Oxford Translational Myeloma Centre (OTMC), Nuffield Department of Orthopedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
2. K36 Therapeutics, Inc. Cambridge, MA, USA
3. Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA
4. Harvard Medical School, Boston, MA
5. Department of Data Science, Dana Farber Cancer Institute, Boston, MA
6. Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

Background: In t(4;14) multiple myeloma (MM), the histone methyl transferase NSD2 gene is placed under the control of the IgH super-enhancer, leading to its overexpression and abnormally high levels of dimethylation of histone 3 at lysine 36 (H3K36me2). High NSD2 promotes MM cell growth, proliferation and cell-cell/cell-matrix adhesion in the bone marrow (BM) microenvironment. KTX1001 is an oral, small-molecule NSD2 inhibitor being evaluated in a Phase 1 trial in late stage MM (NCT05651932; Bories ASH 2024). We report initial characterization of KTX1001 in MM cell lines and biomarker analysis from patient derived BM samples. We find KTX1001's role in disrupting adhesion of MM cells by regulating CD44, CD56 and N-cadherin levels and driving synergy with carfilzomib (CFZ) in a bortezomib-resistant highly adherent cell line.

Methods: KMS11 wildtype (WT) and bortezomib-resistant (BTZ) cells were treated with increasing doses of KTX1001, and proliferation and proportion of adherent cells were quantified. Matrigel assays assessed changes in suspension cells at days 7 and 11. Cell viability and synergy with CFZ were evaluated by CellTiterGlo. Adherent and suspension fractions of KMS11 WT cells were cultured separately and subjected to CFZ dose-response assays. Colony formation assays evaluated KTX1001's effect on cell-cell interaction and colony formation. Gene and protein expression were quantified by RNASeq and Western. Patient samples were analyzed by mass cytometry.

Results: KTX1001 monotherapy treatment of KMS11 WT/BTZ cells resulted in dose- and time-dependent reduction in cell adhesion, with a concomitant increase in suspension cells. While overall cell viability remained unaffected, colony formation was impaired. Transcriptomic profiling of non-adherent KMS11/BTZ cells after KTX1001 treatment revealed differentially reduced expression of adhesion-related genes including CD44, CD56 and TWIST1. Analysis of BM from KTX1001 treated patients demonstrated reduced expression of CD44, CD56, and H3K36me2 in MM cells at Cycle 2.

KTX1001+CFZ combination treatment synergistically inhibited viability in WT and BTZ cells after four days. Transcriptomic analysis of KMS11/BTZ cells treated with KTX-1001+CFZ led to downregulation of adhesion-related genes, especially CD44 and N-cadherin. Additionally, KMS11 suspension cells were significantly more sensitive to CFZ than their adherent counterparts. Suspension cells pre-treated with KTX1001 exhibited enhanced sensitivity to CFZ versus untreated suspension cells.

Conclusions: Inhibition of NSD2 by KTX1001 led to disruption of adhesive properties of MM cells that were mediated by CD44 and N-Cadherin in cell lines and patient samples. Further, the effects of KTX1001 on adhesion sensitized BTZ-resistant cells to combination treatment with CFZ. Analysis of patient samples from the ongoing trial that includes combination of KTX1001+CFZ would help ascertain synergy and the proposed molecular mechanism in clinic.

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