Identification of a First-In-Class SETD2 inhibitor that shows potent and selective anti-proliferative activity in t(4;14) multiple myeloma

t(4;14) multiple myeloma cells are dependent on both H3K36 di and tri-methylation.

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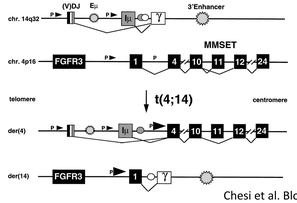
1. Background

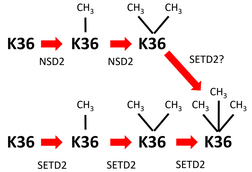
- t(4;14) chromosome translocations are found in 15% of newly diagnosed multiple myeloma (MM) cases and are associated with high risk.
- MM cells with t(4;14) over-express the histone methyltransferase (HMT), WHSC1/MMSET/NSD2, which leads to deregulation of gene expression due to increased di-methylation of Histone H3 at lysine 36 (H3K36me2).
- SETD2 is the only known enzyme capable of tri-methylation of H3K36 and has been reported to play a role in transcriptional elongation and alternative splicing.
- Through our drug discovery efforts, we identified Compound 2, a potent and selective inhibitor of SETD2 with low nM cell biochemical activity and broad selectivity against a panel of other HMTs.
- Inhibition of SETD2 resulted in reduced global tri-methylation of H3K36 in t(4;14) bearing MM cell lines. In contrast, there was no effect on global di-methyl H3K36 levels, indicating that WHSC1 activity is not affected by SETD2 inhibition.
- t(4;14) MM cell lines are uniquely sensitive to SETD2 inhibition.
- These findings show that t(4;14) MM cell lines require SETD2 activity for survival, suggesting that SETD2 inhibitors are strong candidates for the treatment of this high risk subgroup of MM.
- The current chemical series represented by Compound 2 is potent, selective, orally available, and currently under further evaluation for its therapeutic potential.

The t(4;14) chromosome translocation leads to over-expression of WHSC1/MMSET/NSD2 in multiple myeloma.

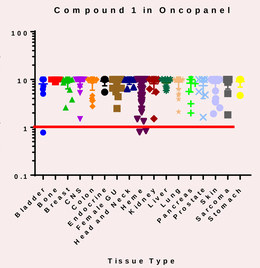


Chesi et al. Blood 1998

WHSC1/MMSET/NSD2 and SETD2 are both H3K36 methyltransferases. SETD2 is the only enzyme capable of generating H3K36me3.

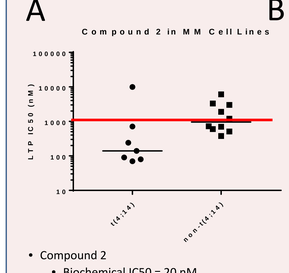


SETD2 inhibitors show selective activity in t(4;14) multiple myeloma cell lines



- Compound 1
  - Biochemical IC50 = 24 nM
  - Cell Methylation IC50 = 83 nM

Figure 2. Compound 1, an inhibitor with similar characteristics to Compound 2, shows little activity in the 280 cell line Eurofins Oncopanel. Cell lines were treated with a 10 point dose curve for 11 days.



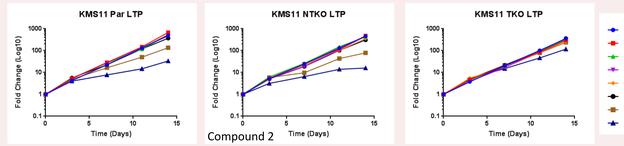
B

Cell Line	TL	Proliferation IC50 (uM)
LP-1	t(4;14)	0.07
KMS-34	t(4;14)	0.08
OPM-2	t(4;14)	0.09
KMS-11	t(4;14)	0.16
NCI-H929	t(4;14)	0.24
EJM		0.38
Delta-47		0.51
HuNS1		0.6
KMM-1	t(8;14)	0.7
KMS-26	t(4;14)	0.71
PCM6		0.72
MM.1R	t(4;16)	1.2
KMS-12-BM	t(11;14)	1.9
MM.1S	t(4;16)	3
RPMI 8226	t(14;16)	3.3
U266B1	t(11;14)	6.1
KMS-28-BM	t(4;14)	10

- Compound 2
  - Biochemical IC50 = 20 nM
  - Cell Methylation IC50 = 50 nM

Figure 3. Compound 2 shows potent anti-proliferative effects in multiple myeloma cell lines. A) Each point represents a cell line. Cell lines are grouped by t(4;14) status. 14-day proliferation IC50 is shown on the y-axis. B) MM cell lines tested in assay with translocation status.

The dependence of t(4;14) MM on SETD2 is related to expression of WHSC1/MMSET/NSD2



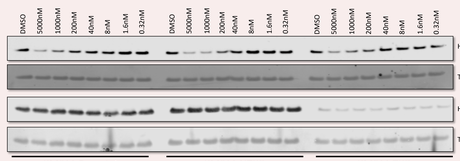
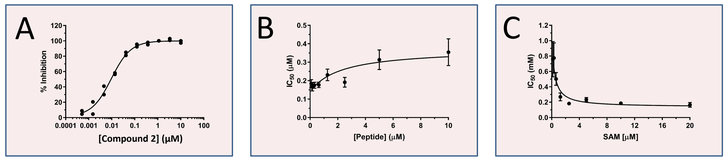


Figure 5. Sensitivity to SETD2 inhibitor is associated with WHSC1/NSD2 over-expression. Two isogenic variants of KMS11 cells, the TKO and NTKOs have been characterized previously (Kuo AJ et al. Mol. Cell. 2011). NTKO cells express only the translocated NSD2. TKO cells express only the non-translocated allele of NSD2. A) 14-day proliferation assay with Compound 2. B) H3K36me2 and H3K36me3 western blots from Parental, TKO and NTKO cell lines treated with Compound 2.

Development of SETD2 inhibitors



D

Enzyme Assay	Compound 2 IC50 (μM)
SETD2	0.02
WHSC1/NSD2/MMSET	>200
SETD7	>200
SETDB1	>200
DOT1L	>200
EHMT2	>200
EZH2	>200
EZH1	>200
SMYD2	>200
SMYD3	>200
PRMT3	>200
PRMT6	>200
PRMT7	>200
PRMT8	>200

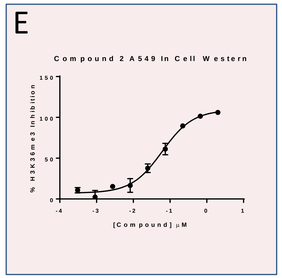
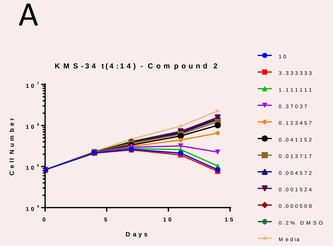
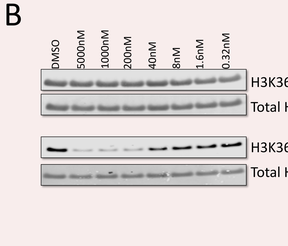


Figure 1. Discovery of potent, selective and cell active inhibitors of SETD2. A) Compound 2 inhibits the biochemical activity of SETD2 with an IC_{50} of 20 nM. Biochemical inhibition of SETD2 by the related compound, Compound 3, is B) noncompetitive with respect to peptide (K_i = 140 nM, αK_i= 370 nM) and C) mixed uncompetitive with respect to SAM (K_i = 1,150 nM, αK_i = 140 nM). D) Compound 2 shows no measurable inhibition of any other HMT tested, including related family member NSD2. E) Compound 2 inhibits H3K36 tri-methylation in A549 cells as measured by an In Cell Western assay measuring H3K36me3.

Cytotoxic effect of SETD2 inhibitors in t(4;14) MM is on target





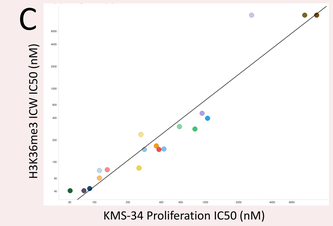
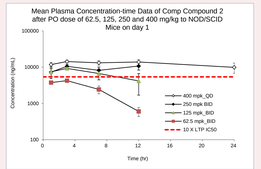


Figure 4. Effects of Compound 2 on a t(4;14) MM cell line are due to SETD2 inhibition. A) KMS-34, a t(4;14) multiple myeloma cell line has a cytotoxic response to Compound 2 with an 80 nM proliferation IC50 in a 14-day assay. B) H3K36 tri-methylation is inhibited with Compound 2 while H3K36 di-methylation is unaffected. C) Strong SAR relationship observed with SETD2 inhibitors when comparing H3K36me3 inhibition potency and anti-proliferative potency. Each point represents a SETD2 inhibitor run in an A549 H3K36me3 assay and a KMS-34 proliferation assay.

Compound 2 is tolerated at effective concentrations in mice and shows PD activity in vivo



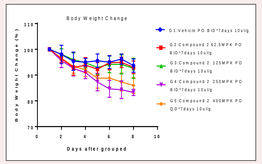
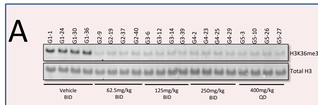


Figure 6. Drug exposure and body weights for 7 day dose range finding study. Compound 2 is tolerated and can be maintained at concentrations that are effective in vitro. A) Levels of Compound 2 are maintained that exceed 10X the KMS11 proliferation IC50 in mice with either BID or QD dosing. B) No body weight loss is observed at 62.5 and 125 mg/kg BID when compared with vehicle control.



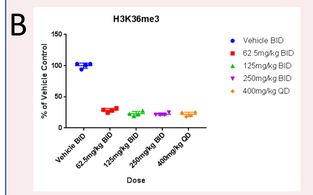


Figure 7. Histones isolated from mouse bone marrow following 7 days of treatment with Compound 2. A) Western blot of histone H3 probed with the H3K36me3 or total H3 antibodies (Cell Signaling). B) Quantitation of western blot. Substantial decrease in H3K36 methylation observed at all doses of Compound 2

6. Conclusions

- We have identified a first-in-class SETD2 inhibitor, Compound 2. It is potent, selective and orally bioavailable.
- Compound 2 shows selective sensitivity in t(4;14) multiple myeloma cell lines.
- Sensitivity of t(4;14) cell lines to Compound 2 is dependent on WHSC1/MMSET/NSD2, another H3K36 methyltransferase over-expressed in t(4;14) MM.
- Compound 2 has PK properties that allow it to exceed 10X the proliferation IC50 of t(4;14) MM cell lines in rodents.
- H3K36me3 is modulated in mouse bone marrow following exposure to Compound 2.