A small molecule inhibitor of the RNA m⁶A writer METTL3 inhibits the development of AML *in vivo*

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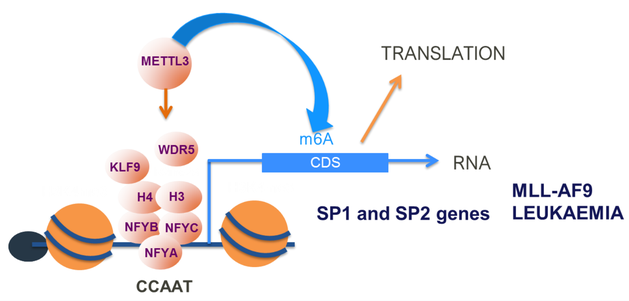
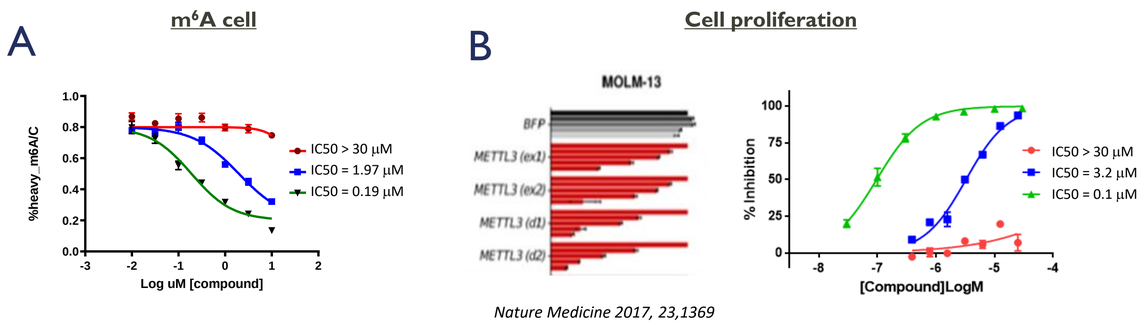
Abstract

METTL3 is an RNA methyltransferase which is responsible for the deposition of N-6-methyladenosine (m⁶A) on mRNA targets such as SP1, to modulate their stability and expression. METTL3 was identified as an essential gene for the growth of AML cells and proposed as a novel target for cancer therapy (Barbieri 2017). We present the *in vitro* and *in vivo* characterization of novel small molecule inhibitors of METTL3, which recapitulate the genetic validation of METTL3 as a novel cancer target using a pharmacological audit trail.



Cellular activity



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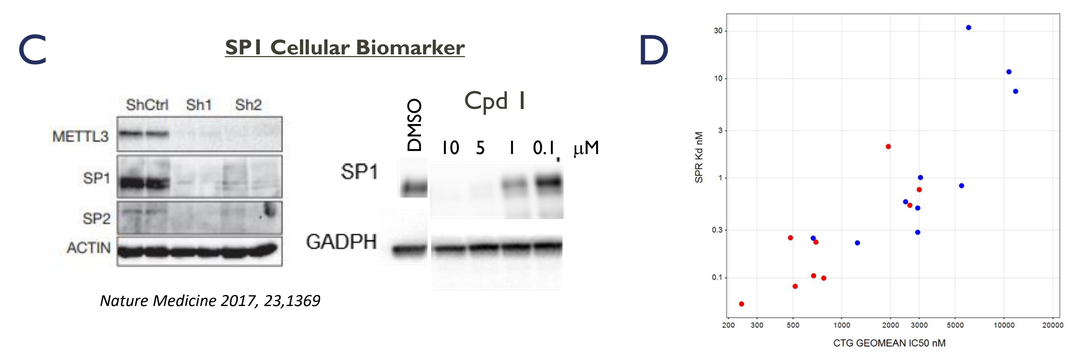

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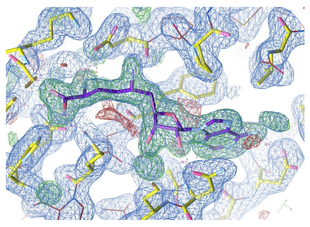
A. Inhibition of m⁶A by compound 1 (blue), lead compound (green), inactive analogue (red)
B. Anti-proliferative effect of METTL3 depletion or compound inhibition in MOLM13 cells
C. Inhibition of SP1 expression after METTL3 depletion and inhibition by compound 1
D. Correlation of METTL3 binding affinity (SPR) with cell potency (CTG) for examples from two chemical series

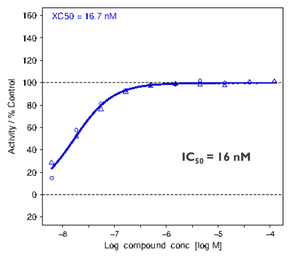
Characterisation of METTL3 inhibitors in orthogonal systems

A Crystallography

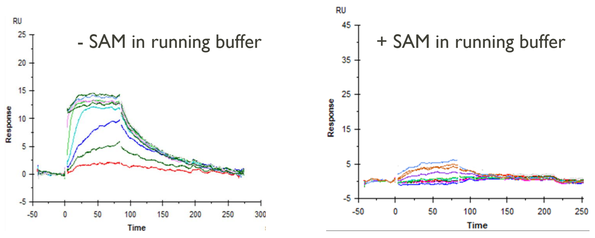


- Structure based drug design driven compound optimization
- Soakable METTL3/14 complex crystal system – example sinefungin here
- > 30 high resolution crystal structures solved across multiple chemical series

B Biochemical assay

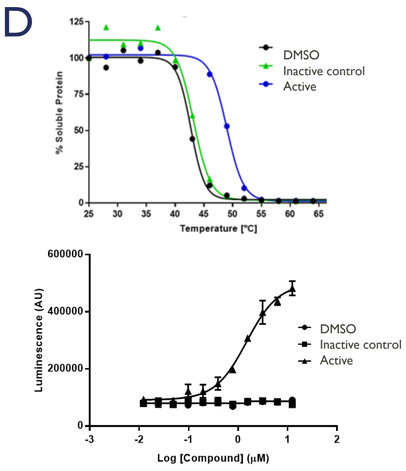


C Surface Plasmon Resonance



- Confirmation of binding to METTL3
- SAM competition experiments
- Binding kinetics determination (K_{on}/K_{off})

D Target engagement assay



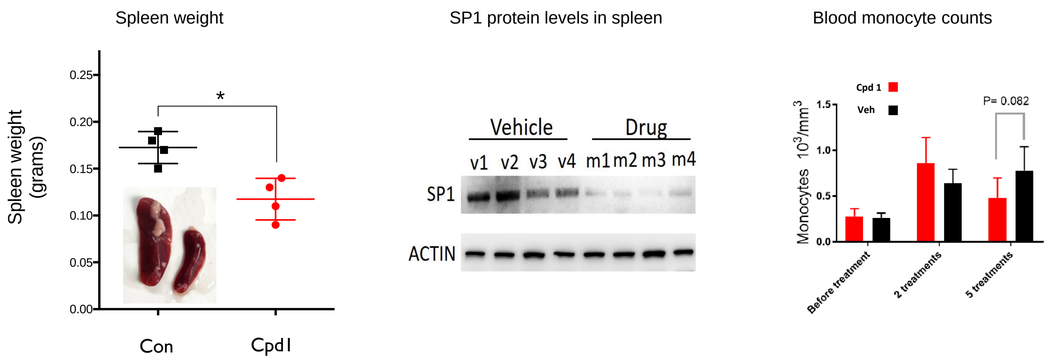
- Thermal stabilisation of METTL3 protein in cells on compound binding

E

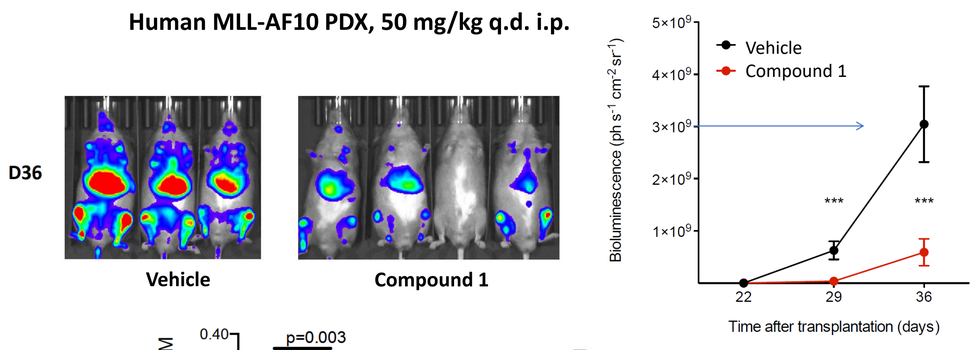
Assay	Sinefungin	HTS Hit	Cpd 1 (PoC)	Cpd 2 (Lead)
METTL3 SPR Kd	0.454	(1)	0.003	0.000055
METTL3 Biochemical assay IC_{50}	1.2	5.4	0.016	<0.006
METTL3 InCELL pulse EC_{50}	(>25)	>25	4	0.216
MOLM13 Cell SP1 EC_{50}	>25	>25	0.97	0.022
MOLM13 Cell antiproliferation EC_{50}	>25	>25	9	0.190

Compound 1 is efficacious in physiologically relevant AML models *in vivo*

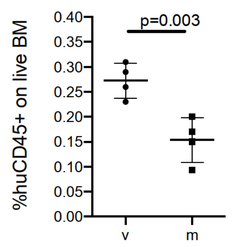
A Murine primary MLL-AF9 AML, 30 mg/kg q.d. i.p.



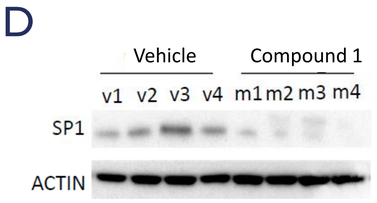
B Human MLL-AF10 PDX, 50 mg/kg q.d. i.p.



C



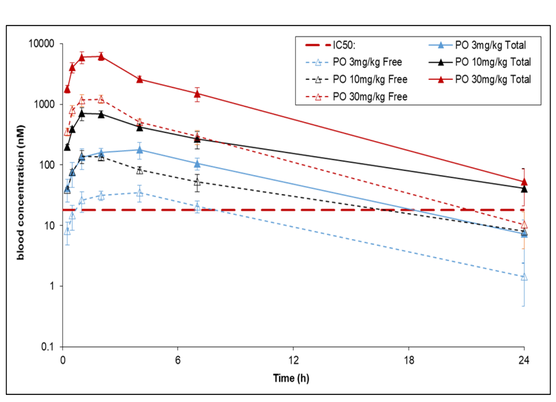
D



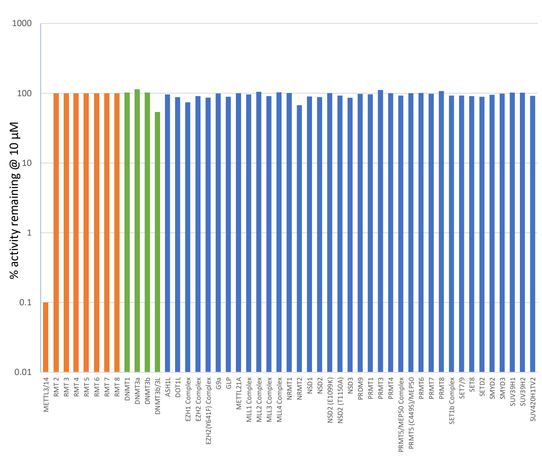
A. Inhibition of murine MLL-AF9-driven leukemia by compound 1 *in vivo*. Intra-peritoneal (IP) administration of 30 mg/kg compound 1 daily for five days leads to significant reduction in tumour growth.
B. Inhibition of PDX model of human MLL-AF10 driven leukemia by compound 1 *in vivo*. Intra-peritoneal (IP) administration of 50 mg/kg compound 1 daily for 21 days leads to significant reduction in tumour growth.
C. Reduction in leukemia cells in bone marrow after 2 weeks treatment
D. Inhibition of SP1 and Brd4 biomarkers in spleen after 2 weeks treatment

Suitability for *in vivo* proof of concept studies

A Pharmacokinetics



B Selectivity



A. *In vivo* pharmacokinetics (PK) data from oral administration of compound 1 in mice showing excellent oral bioavailability and exposure. Mean total and free blood concentrations of compound 1 following oral (PO) administration to male C57Bl/6J mice at 3, 10 or 30 mg/kg

B. Compound 1 is highly selective for METTL3 inhibition against a panel of RNA (orange), DNA (green) and protein (blue) methyltransferases

Summary

We have described the comprehensive characterization of potent and selective inhibitors of the METTL3 RNA methyltransferase, and demonstrated their activity and utility using biochemical, cellular and *in vivo* systems. We have demonstrated that inhibition of METTL3 by small molecules *in vivo* leads to a pronounced anti-tumor effect in a physiologically relevant model of acute myeloid leukemia.

Harnessing the Power of RNA Epigenetics

Chemistry, protein purifications, high-throughput screens and PK studies performed by evotec