FIRST-IN-CLASS TARGETED PROTEIN DEGRADER OF MLLT1/3 FOR THE TREATMENT OF ADVANCED AML AND ALL INCLUDING MENIN INHIBITOR RESISTANT/REFRACTORY DISEASE

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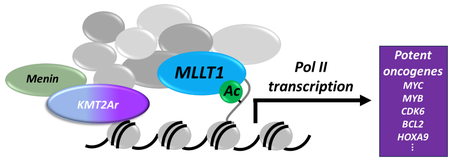
INTRODUCTION

MLLT-1 and -3 are highly homologous histone reader proteins that are critical mediators of the Super Elongation Complex (SEC), a developmental regulator of transcription.[1]

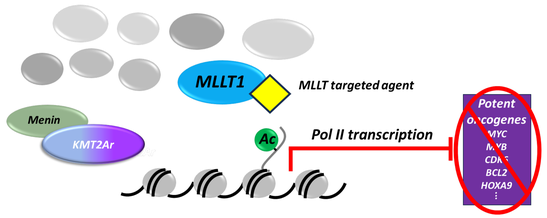
The SEC, which is commonly hijacked in cancer, promotes RNA Polymerase II dependent transcription of major oncogenes and tumour drivers e.g., *MYC*, *BCL2*, *CDK6*, *HOXA9* and *MEIS1*. SEC-dependent transcription is a recognized driver in multiple AML and ALL sub-populations, such as those defined by *KMT2A*-rearrangements (KMT2Ar) and NPM1 mutations (NPM1m).[2,3]



Schematic of the SEC in KMT2Ar leukemia

Clinical proof-of-concept for targeting the SEC has recently been demonstrated with inhibitors of menin, a protein associated with the SEC in some dependent cancers. However, many patients do not respond to, or rapidly progress on menin inhibitors. Hence there is a need for therapies that can broadly target SEC-dependent cancers including those resistant to menin inhibitors.[4,5]

Targeting MLLT1/3 represents a novel approach to blocking the SEC and MLLT1/3 inhibitors have shown anti-cancer activity in a range of AML and ALL cell model systems. Given the central role MLLT1/3 plays in the SEC, we sought to establish whether inhibition or degradation of MLLT1/3 was the optimal therapeutic mechanism and to design an oral MLLT1/3 targeted agent as a first-in-class pan-SEC drug.

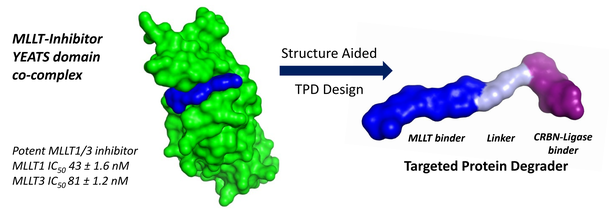


MLLT1/3 targeting disrupts SEC complexes on transcription start sites

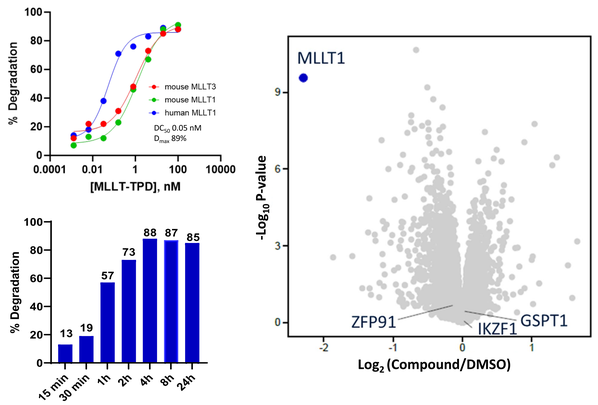
REFERENCES: 1. *Cell Mol Life Sci* (2018) **75**, 3931-3941: 2. *Nat Rev Mol Cell Biol* (2012) **13**, 543-547: 3. *Cancer Discovery* (2023) **13**, 724-745: 4. *Nature* (2023) **615**, 913-919: 5. *Nature* (2023) **615**, 920-924: 6. *Science Advances* (2024) **10**, eado1432.

RESULTS

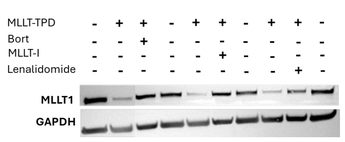
1. Discovery of highly potent and selective MLLT1/3-Targeted Protein Degrader (TPD)



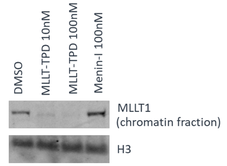
Potent, rapid and selective degradation of MLLT (whole cell lysates)



Degradation of MLLT is proteasome- and CRBN-dependent



Chromatin bound MLLT is degraded



MLLT degradation is more effective than inhibition against AML and ALL cells

KMT2Ar cell lines	MLLT-I IC_{50} nM	MLLT-TPD IC_{50} nM
MV4:11 (MLL-AF4)	290	30
SEM (MLL-AF4)	27000	70
MOLM-13 (MLL-AF9)	1800	120
ML2 (MLL-AF6)	6100	960

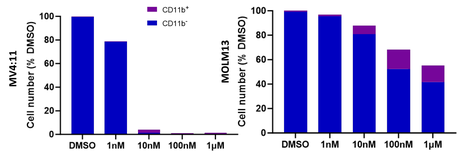
MLLT1/3 YEATS domain inhibition measured by an HTRF assay. Unless otherwise stated experiments were run in MV4:11 cells. Degradation of human MLLT1 determined at 4h. Degradation of mouse MLLT1 and 3 determined at 5h in NIH-3T3 cells. Kinetics of degradation performed with 100nM MLLT-TPD. DC_{50} & kinetics determined using JESS Protein Simple. DIA mass spec. global proteomics used to assess selectivity at 10nM (4h) MLLT-TPD. Bortezomib used as a proteasome inhibitor, Lenalidomide used as a CRBN binder. MLLT-I is an in house proprietary MLLT1/3 inhibitor, closely related to MLLT-TPD. AML/ALL cell viability measured in Elplasia plates by Cell-TiterGlo readout (5d). MLLT-TPD used for all experiments except for chromatin MLLT1 degradation (close MLLT-TPD analog, DC_{50} 1.4nM) and AML/ALL cell viability (second close MLLT-TPD analog. DC_{50} 10nM).

2. MLLT-TPD has a potent and broad impact across AML and ALL cell lines

Driver	Cell line	IC_{50} nM
KMT2Ar	MV4:11 (KMT2A-AF4)	0.8
	SEM (KMT2A-AF4)	3.1
	MOLM-13 (KMT2A-MLLT3)	33
	ML2 (KMT2A-AF6)	10
NPM1m	OCI-AML3	24
Non-cancer	HEK	>10000

Driver	Cell line	IC_{50} nM
Other	697 (E2A-PBX1)	4.1
	REH (ETV6-RUNX1)	45
	Kasumi-1 (RUNX1-RUNX1T1)	42
	SKNO1 (RUNX1-RUNX1T1)	39
	U937 (PICALM-MLLT10)	>1000
	K562 (CML)	>1000

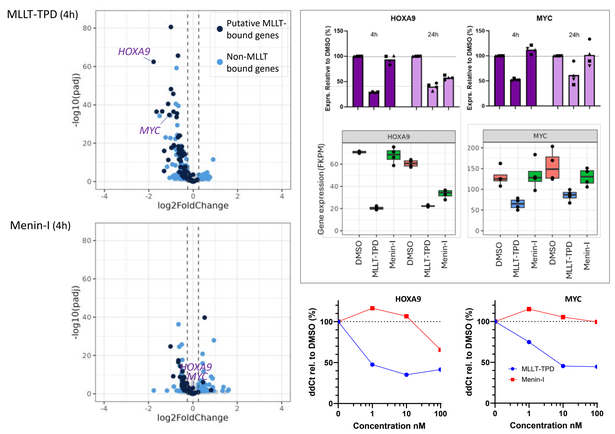
MLLT-TPD reduces cancer cell viability and relieves differentiation block



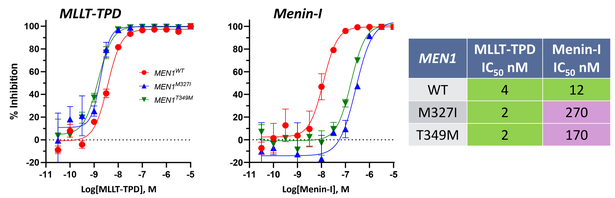
Cell viability measured by Cell Titer-Glo (12d). Differentiation measured by flow cytometry staining for CD11b+ cells (8d). Cell number measured by flow cytometry counting beads (8d).

3. MLLT-TPD has a differential profile to menin-inhibitors

MLLT-TPD drives rapid and broad inhibition of SEC target gene transcription



MLLT-TPD retains activity in clinically relevant menin inhibitor resistant cell lines

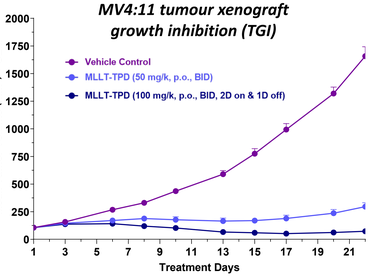


MEN1	MLLT-TPD IC_{50} nM	Menin-I IC_{50} nM
WT	4	12
M327I	2	270
T349M	2	170

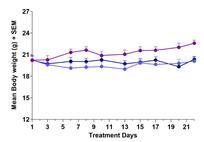
RNA-seq experiment performed with MLLT-TPD (10nM, 4h and 24h) and Menin-I (revuminib, 100nM) in MV4:11 cells. Reads mapped using Salmon pseudo-alignment. Volcano plots highlight differentially expressed transcripts. Putative MLLT1 bound genes (dark blue) defined based on high MLLT1 binding at gene promoters, using ChIP-seq data.[6] qPCR data and expression calculated as FPKM (Fragments Per Kb of transcript per Million reads) from the RNA-seq analysis shown for *HOXA9* and *MYC* transcripts. Independent concentration-response qPCR data with MLLT-TPD and Menin-I treatment (24h). Activity of MLLT-TPD and Menin-I on wildtype MV4:11 cells plus MEN1 M327I and T349M edited MV4:11 cell pools assessed by CellTiter-Glo (8d). MLLT-TPD used for all experiments except for menin resistance studies (close MLLT-TPD analog, DC_{50} 1.4nM).

4. Oral MLLT-TPD is well tolerated and drives tumour regression with no evidence of heme toxicity in mouse

MV4:11 tumour xenograft growth inhibition (TGI)

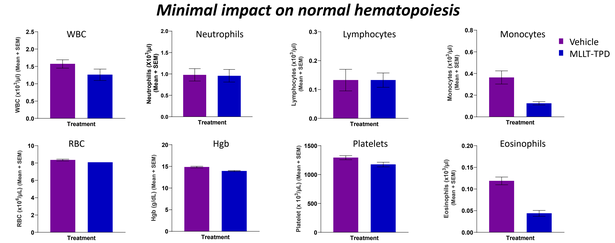


Well tolerated (body weight)

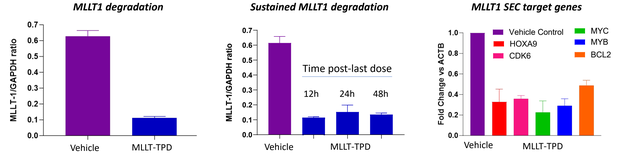


MLLT-TPD degrades both murine MLLT1 and MLLT3 (Panel 1)

Minimal impact on normal hematopoiesis



PD studies show MLLT degradation and SEC gene transcription inhibition in tumours



In vivo work performed in NOD-SCID mice inoculated with 5×10^6 MV4:11 cells/mouse. Efficacy Study: Compound treatment initiated when tumours measured 80-150mm^3 according to outlined dosing regimen. Cell blood counts measured by flow cytometry at the end of the study. PD study: MLLT-TPD (100mg/kg p.o. BID) dosing initiated when tumours measured 300-450mm^3. MLLT degradation and SEC target gene expression assessed in tumours after 3d dosing (24h post last dose). Sustained MLLT degradation assessed in tumours after 2d dosing at the times indicated post last dose.

CONCLUSIONS

- First-in-class oral, selective, TPD's of MLLT1/3 identified
  - More effective in cells than related MLLT inhibitors
- MLLT-TPDs have potent and broad activity against a panel of AML and ALL cell lines
  - Rapid and sustained abrogation of SEC target gene transcription including multiple potent oncogenes
- MLLT-TPDs have a differentiated profile vs menin inhibitors including activity in menin inhibitor resistant cell models
- MLLT-TPDs are highly effective in a mouse model of AML from oral dosing
  - Well tolerated with no impact on normal hematopoiesis

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