Characterizing Antitumor Responses to EXS74539, a Novel, Reversible LSD1 Inhibitor with Potential in Small-cell Lung Cancer

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INTRODUCTION

Lysine demethylase 1 (LSD1) is a potentially important target in oncology due to its role in histone demethylation and consequent suppression of the expression of genes required for cellular differentiation. High LSD1 expression is associated with downregulated differentiation pathways in several tumor types, including hematological, breast, lung and prostate cancer, as well as small-cell lung cancer (SCLC) which is also associated with a high incidence of brain metastasis. Although selective LSD1 compounds are in clinical development, understanding of the molecular markers of tumor sensitivity to inhibition of LSD1 is incomplete. Exploration of LSD1 target inhibition has been restricted by on-target dose-limiting toxicities, including thrombocytopenia, and the lack of central nervous system (CNS) penetration in LSD1 clinical compounds in development for SCLC.

Here, we report on the pharmacological characterization of EXS74539 ('539), a potent, selective and reversible LSD1 inhibitor with CNS penetration.

METHODS

'539 was characterized in a range of preclinical pharmacological models and ADMET studies.

- '539 was shown to have CNS penetration across a range of preclinical species (data not shown)
- '539 anti-proliferative activity on the SCLC cell lines NCI-H1417 and NCI-H69 (*ASCL1* subtype) and the NCI-H526 cell line (*POU2F3* subtype) determined using CellTiter-Glo® or measurement of cell confluence (Incucyte®) after 10 days of treatment
- *In vivo* studies: xenografts were grown subcutaneously in female SCID mice for the NCI-H69 model, and BALB/c mice for the NCI-H1417 and NCI-H526 models
- '539 effect on a panel of LSD1 target genes assessed by reverse transcription-quantitative PCR (RT-qPCR). *In vitro*, RNA samples were isolated following cell exposure to '539 for 96 hours. Gene expression of selected LSD1 target genes quantified in tumor samples collected from efficacy studies at study termination
- Platelet numbers measured in mouse or rat blood using ProCyte Dx Analyzer (IDEXX)

RESULTS

Table 1: '539 is a highly differentiated LSD1 inhibitor with a unique property profile

	Assay	Candidate Properties	Competing Irreversible Ph 1/2 Candidate	Competing Reversible Candidate	EXS74539
CNS penetration	Brain:plasma ratio	>0.5			
Target affinity and mechanism	LSD1 IC_{50} (nM)	<10			
	Surface plasmon resonance	Reversible			
Cell potency and *in vivo* efficacy	SCLC cell line proliferation (nM)	<100			
	Efficacy in 2x SCLC models *in vivo*	TVR >65%			
Safety and metabolism	CV safety margin				
	Human microsome Clint µL/min/mg	<15			
	Human hep Clint µLmin/10^6 cells	<15			
Permeability / transporter liability	MDCK-MDR1 efflux ratio (Pgp inhibition)	<2			
	Solubility pH 7.4 µg/ml	>50			
PK properties	F % (p.o.)	>30%			
	Half-life	Short acting			

- CNS penetrant
- Potent and reversible
- Highly selective (including related amine oxidases)
- Efficacious *in vivo*
- Excellent metabolic stability, bioavailability and no efflux
- Shorter predicted half-life than competitors

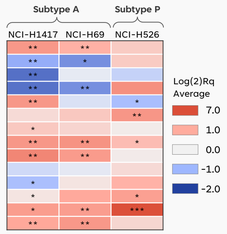
Meets or exceeds criteria; Minor deviation; Major deviation; Not tested

Table 1: Preclinical profile of '539 compared to clinical irreversible and reversible examples.

Table 2: Similar anti-proliferative activity of '539 observed across SCLC cell lines

SCLC Cell Line	SCLC Subtype	'539 Proliferation IC_{50} (nM; N, 95% CI)
NCI-H1417	*ASCL1*	28 (10, 19-41)
NCI-H69	*ASCL1*	12 (14, 10-14)
NCI-H526	*POU2F3*	11 (8, 8-14)

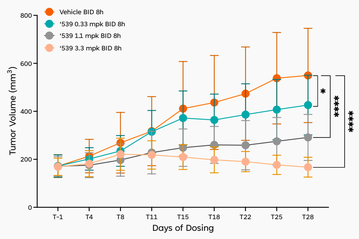
Figure 1: Modulation of LSD1 target genes by '539 in SCLC cell lines *in vitro*



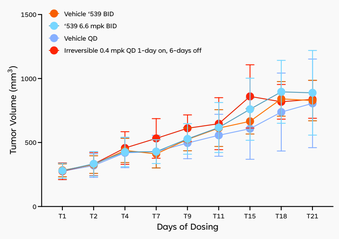
- Exposure of SCLC cell lines to '539 at 0.3 µM for 96 h resulted in modulation of LSD1 target genes
- Different responses were observed between the subtype A cell lines and between subtypes A and P
- P-value calculated by performing a t-test with adjusted FDR (false detection rate); 0.05<*<0.01; 0.01<**<0.001; 0.001<***

Figure 2: *In vitro* sensitivity alone does not predict *in vivo* response; combining transcriptional and functional responses *in vitro* may do so

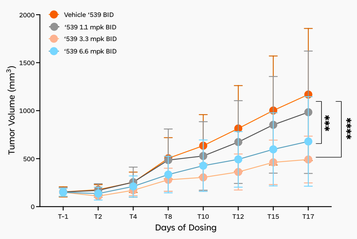
A) Tumor growth inhibition study in NCI-H1417 xenograft model



B) Tumor growth inhibition study in NCI-H69 xenograft model



C) Tumor growth inhibition study in NCI-H526 xenograft model

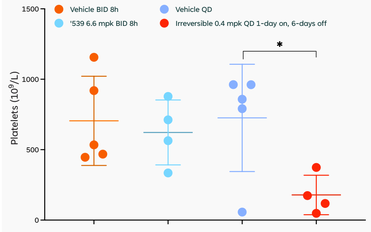


D) Tumor-derived transcriptional changes associated with exposure to '539

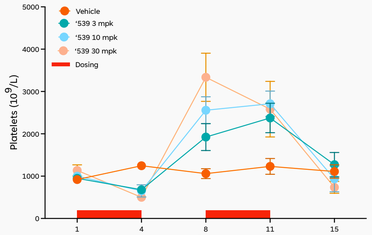
Figure 2: '539 or an irreversible inhibitor were administered orally to mice bearing **(A)** NCI-H1417, **(B)** NCI-H69, and **(C)** NCI-H526 tumors as indicated and tumor volume determined (mean +/- SD). Statistical significance evaluated via two-way ANOVA with Dunnett's multiple comparison test (*P≤0.05; ***P<0.001; ****P<0.0001). **(D)** Tumors from the efficacy studies were collected at study termination and RNA extracted for RT-qPCR analysis of LSD1 target genes. Data shown for top dose groups for each study. Changes in LSD1 target gene expression in SCLC tumor samples at study termination. P-value calculated by performing a t-test with adjusted FDR (false detection rate); P-value: 0.05<*<0.01; 0.01<**<0.001; 0.001<***

Figure 3: Shorter half-life and reversibility may benefit on-target toxicity management; intermittent dose regimen maintains efficacy

A) Platelet count in mouse efficacy study in NCI-H69 xenograft model



B) Reversible platelet modulation in rat study (3-days on, 4-days off)



C) Tumor growth inhibition study in NCI-H1417 xenograft model

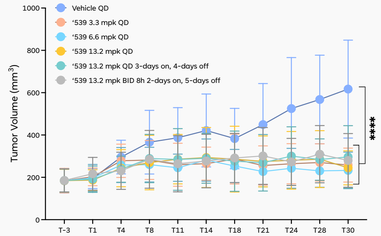


Figure 3: (A) Platelet numbers were determined in mouse blood following administration of LSD1 inhibitors for 21-days in an efficacy study (see **Figure 2B**). **(B)** Platelet numbers were determined in rat following administration of '539 in an intermittent dose regimen in a 2-week study. **(C)** '539 was administered orally to mice bearing NCI-H1417 with the dose regimen indicated.

DISCUSSION

The reversible, potent and CNS penetrant LSD1 inhibitor, '539, inhibits SCLC cell line proliferation and shows *in vivo* efficacy. Characterizing SCLC cell line sensitivity *in vitro* and *in vivo* identified gene fingerprints that may have use as markers of sensitivity, which we are currently characterizing and validating in human SCLC patient samples. The reversible mechanism-of-action and suitable half-life may provide an opportunity to better manage on-target dose-limiting thrombocytopenia.

CONCLUSIONS

- '539 is a potent, highly selective LSD1 inhibitor combining reversibility and brain penetrance
- A suitable therapeutic index has been established with no unexpected toxicity in non-GLP studies
- '539 has potential in a broad range of hematologic and oncologic diseases as either a monotherapy or combination therapy
- Translational work will define optimal patient populations and validation of PD biomarkers

Our LSD1 inhibitor, EXS74359, has been precision-designed to have a unique property profile and address the target optimally in the patient population.

REFERENCES

1) Rudin et al. Nat Rev Cancer, 2019

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