Abstract #207

Discovery of D3S-001, a highly potent and CNS-penetrant inhibitor of KRAS G12C with rapid and sustained target engagement kinetics

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Background

Covalent inhibition of mutant KRAS G12C protein by locking this oncogenic driver in its inactive state is clinically efficacious. Sotorasib and adagrasib have improved overall response rates (ORR) and progression-free survival (PFS) in patients with KRAS G12C -mutant non-small cell lung cancer (NSCLC). However, achieving deeper and more durable responses remain as challenges in targeting KRAS G12C. Treatment of brain metastasis in NSCLC remains a high unmet medical need.

D3S-001, currently in a phase 1 trial (NCT05410145), is designed to improve clinical outcomes by optimizing KRAS G12C target engagement (TE). Here we report its preclinical activity, selectivity, central nervous system (CNS) penetrant properties, and predicted target inhibition at dose levels under phase 1 study.

Material and methods

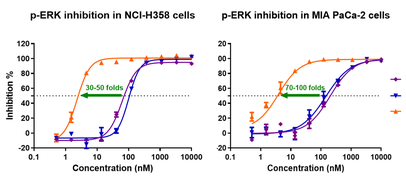
The kinetics of KRAS G12C TE was measured by GTP-RAS ELISA in tumor cells and xenografts. Covalent adduct formation with KRAS G12C protein was confirmed by LC-MS. D3S-001 selectivity was assessed by free-cysteine proteomics. Anti-proliferative effect on cancer cells was evaluated by the CTG assay. Cancer cell line- and patient-derived xenografts were utilized for *in vivo* efficacy studies. D3S-001 pharmacokinetics (PK) in plasma and cerebrospinal fluid (CSF) were investigated in beagle dogs. Human target inhibition was calculated based on human PK prediction, free drug fraction, and *in vitro* TE data.

Overall properties of D3S-001

Properties		D3S-001
Physicochemical Properties	KS (μM) PH=7.4	133.2
	PPB (Unbound %) H / D / C / R / M	4.8 / 3.3 / 7.8 / 4.8 / 1.5
***In vitro* Efficacy**	Biophysical k_{inact}/KI ($M^{-1}s^{-1}$)	1.58E+06
	Cellular k_{inact}/KI ($M^{-1}s^{-1}$)	55,084
	NCI-H358 p-ERK IC_{50} (nM)	2.6
	NCI-H358 proliferation IC_{50} (nM)	4.6
Selectivity	PC9 (KRAS wt) IC_{50} (nM)	>10,000
ADME & Safety	CNS penetration (preclinical, dog)	Kp, uu = 0.67
	hERG IC_{50} (μM)	36.04*
	CYP450 IC_{50} (μM) 1A2/ 2C9/ 2C19/ 2D6/ 3A4	>50 / 18.1 / 28.6 / >50 / >50
Clinical PK	AUC (hr*μg/mL)	3.20 (predicted at 200 mg QD)
	Half life (hrs)	17 (predicted)
	C_{trough} (nM) at steaty state	125 (predicted)
	Free drug at C_{trough} (nM)	6
	Predicted KRAS G12C target engagement at C_{trough}	>95%

Results — *in vitro* and *in vivo* activity

- **D3S-001 demonstrated superior potency in inhibiting KRAS G12C downstream signaling compared with AMG510 and MTRX849**



IC_{50} (nM)	NCI-H358	MIA PaCa-2
AMG510	62.5	203.1
MRTX849	100.8	144.9
D3S-001	2.6	2.5

Figure 1. Inhibitory effects of D3S-001 on phospho-ERK level quantified by HTRF assay in KRAS G12C mutant cancer cell line NCI-H358 and MIA PaCa-2

- **D3S-001 demonstrated strong anti-proliferation effects in KRAS G12C mutant cancer cell lines and no cytotoxic effects in cancer cell lines without KRAS G12C mutation. In whole proteome free-cysteine profiling, D3S-001 demonstrated high selectivity**

Cancer type	Cell line	KRAS status	Anti-proliferation IC_{50} (nM)		
			D3S-001	AMG510	MRTX849
Lung	Calu-1	G12C	1.3	17.5	74.4
Pancreas	MIA PaCa-2	G12C	2.4	29.5	61.1
Colon	SW837	G12C	4.3	113.4	102.9
Lung	NCI-H358	G12C	4.6	67.0	93.7
Bladder	UM-UC-3	G12C	8.9	215.5	274.9
Lung	A549	G12S	> 10000	> 10000	2186.2
Lung	NCI-H441	G12V	> 10000	> 10000	3235.6
Lung	PC-9	WT	> 10000	> 10000	2111.6
Colon	SW480	G12V	> 10000	> 10000	2587.2
Pancreas	AsPC-1	G12D	> 10000	> 10000	2074.7

Table 1. Inhibitory effects of D3S-001 on cell proliferation in KRAS G12C and KRAS non-G12C mutant cancer cell lines determined by 5-day 2D CTG assay

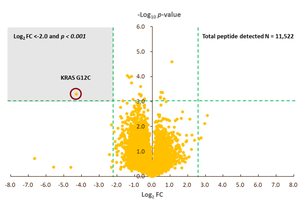
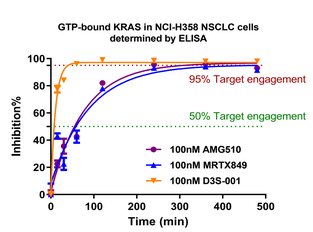


Figure 2. The cysteine12 peptide from KRAS G12C is the only peptide that met the criteria for covalent conjugation detected among over 10,000 cysteine-containing peptides in mass spectrometry-based free cysteine-proteome analysis

- **In cell-based target engagement kinetics analysis, D3S-001demonstrated substantially faster target engagement than AMG510 and MRTX849**



Compound	Time required for 50% TE	Time required for 95% TE
AMG510	55 minutes	305 minutes (5 hours)
MRTX849	58 minutes	446 minutes (7.4 hours)
D3S-001	8 minutes	40 minutes

Figure 3. Target engagement (TE) kinetics of AMG510, MRTX849 and D3S-001 was evaluated by GTP-RAS ELISA in NCI-H358 cell lysates collected at different timepoint after treatment with respective compound at 100nM.

- **D3S-001 demonstrated more efficient target engagement than AMG510 and MRTX849**

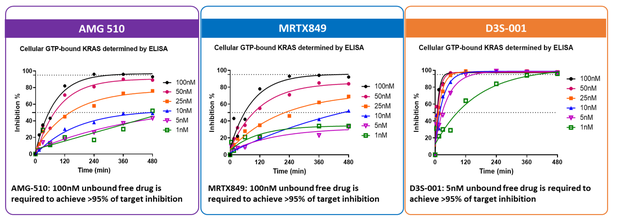


Figure 4. TE kinetics of AMG510, MRTX849 and D3S-001 at indicated concentrations was evaluated by GTP-RAS ELISA in NCI-H358 cell lysates collected at different timepoint after treatment. D3S-001 achieved near complete target inhibition 5 nM; while both AMG510 and MRTX849 require100nM to achieve >95% TE.

- **D3S-001 achieved higher KRAS G12C TE *in vivo and correlatedly led to deeper anti-tumor response in NCI-H358 xenograft model when compared with MRTX849 and AMG510***

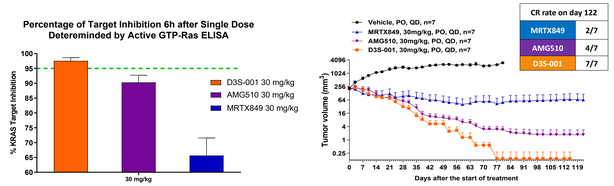


Figure 5. (left) Percentage of KRAS target inhibition was measured by GTP-RAS ELISA using NCI-H358 xenograft tumors harvested 6 hours after a single dose of D3S-001, AMG510 or MRTX849 at 30 mg/kg. (right) Tumor growth in NCI-H358 xenograft model was monitored overtime. 7/7 mice in D3S-001 treatment group achieved complete tumor regression at day 119.Treatment was stopped for all groups at day 122.

- **D3S-001 demonstrated deeper anti-tumor response as a monotherapy or in combination with anti-EGFR antibody in SW837 colorectal cancer xenograft model**

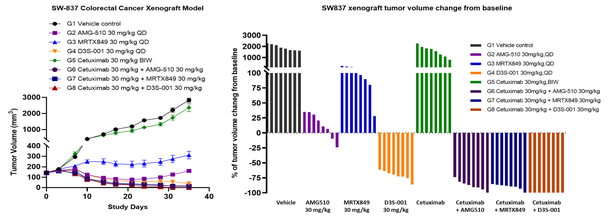
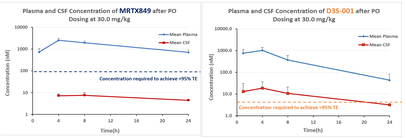


Figure 6. SW837 colorectal cancer xenograft model was treated with AMG510, MRTX849 and D3S-001 as monotherapy or in combination with cetuximab. (left) Tumor growth was monitored overtime. (right) Percentage of tumor volume change from baseline for individual mouse in each group at the end of the experiment.

Results — CNS exposure and efficacy

- **D3S-001 demonstrated significant CNS penetration in preclinical PK studies**



MRTX849 PK study in beagle dogs (n=4*)	C_{max} (nmol/L)	AUC_{0-last} (h*nmol/L)
Plasma	2523	33545
CSF	8.5	142
Kp,uu		0.47

D3S-001 PK study in beagle dogs (n=4*)	C_{max} (nmol/L)	AUC_{0-last} (h*nmol/L)
Plasma	1089	7976
CSF	18.6	177.7
Kp,uu		0.67

*Figure 7. Plasma and cerebrospinal fluid (CSF) exposure of MRTX849 and D3S-001 in beagle dog PK study. *same 4 beagle dogs were used sequentially for the two compounds after washout period.*

- **D3S-001 treatment led to sustained intracranial tumor regression in NCI-H1373^{luc} CNS metastases model**

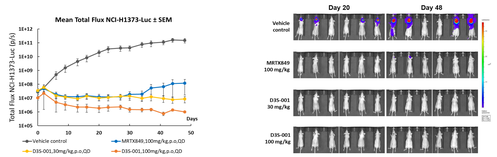
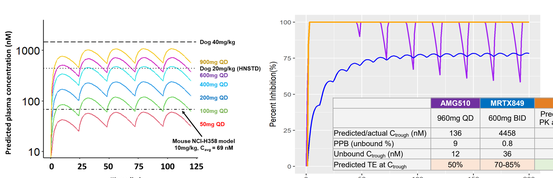


Figure 8. NCI-H1373^{luc} NSCLC cells were injected intracranially. (left) Tumor growth determined by luminescent imaging was monitored overtime. (right) Luminescence images on day 20 and day 48 for treatment groups.

Predicted TE at clinically relevant doses

- **Predicted plasma concentrations of D3S-001 at Phase 1 dose escalation indicate high therapeutic index and sustained complete target blockage**



	AMG510	MRTX849	D3S-001
	960mg QD	600mg BID	Predicted human PK at 200 mg QD
Predicted/actual C_{trough} (nM)	136	4458	125
PPB (unbound %)	9	0.8	4.8
Unbound C_{trough} (nM)	12	36	6.0
Predicted TE at C_{trough}	50%	70-85%	> 95%

Figure 9. D3S-001 dose will be escalated from 50 mg to 900 mg QD. (left) Human PK predication at each cohort during dose escalation. (right) TE in human was calculated based on PK prediction, free drug fraction, and cellular TE data. D3S-001 was predicted to achieve complete TE at 100 mg QD at $C_{average}$ and 200 mg QD at C_{trough}.

Conclusions

- **D3S-001 demonstrated rapid and near complete TE with KRAS G12C *in vitro* and *in vivo*. Its potency, selectivity, and CNS-penetrance support clinical development in patients with KRAS G12C mutation. Optimal TE predicted at clinical exposures may lead to deeper and more durable responses in both systemic and intracranial tumors than currently available KRAS G12C inhibitors.**
- **Phase 1 clinical trial investigating the safety and PK for D3S-001 in advanced solid tumors with KRAS G12C mutation is currently recruiting (NCT05410145).**

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