Route-Dependent Central Effects and Efficacy Prediction of a Nucleic Acid Drug: Comparison of ICV and IT Administration

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Purpose

Limited BBB permeability restricts CNS delivery of nucleic acid therapeutics. ICV and IT administration are commonly used in mice. ICV achieves high and reproducible CNS exposure, whereas IT enables repeated dosing and greater clinical relevance. We compared target gene KD, brain exposure and acute CNS toxicity after single ICV, single IT, and repeated-IT administrations.

Summary

To achieve cortical target engagement, single-dose ICV and IT routes were compared. ICV produced higher exposure and greater KD than IT.
Approximately 4-fold higher IT doses were required for comparable efficacy; however, a 4-fold dose via IT injection is not feasible in terms of toxicity.
Repeated IT dosing (once daily for four consecutive days) achieved cortical exposure and KD comparable to ICV without a marked increasing toxicity.
Plasma concentrations on the dosing day correlated with cortical KD at 14 days, indicating that post-dose plasma monitoring may enable efficacy prediction and guide additional dosing.
These findings broaden nonclinical options for CNS direct administration routes and provide additional strategies for clinical bridging.

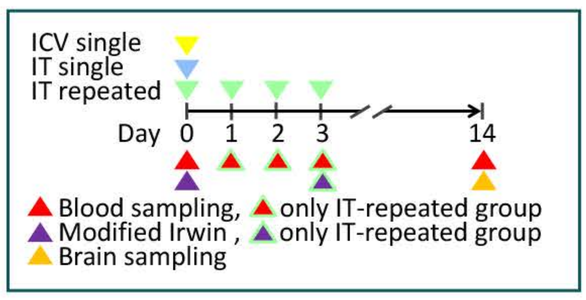
Materials and Methods

ASO

Mouse MALAT1 ASO [1)]
5'-GGGTmCAGCTGCCAATGmCTAG-3'
ASO has fully phosphorothioate backbone. Underlining: 2'-O-methoxyethyl modified base ASO; mC: methylcytosine. ASO was purchased from GENEDESIGN, Ajinomoto Bio-Pharma Services.
1) G. Hung, et.al. 2013. *Nucleic Acid Ther* **23**:6, 369-378.

Animal study

C57BL/6J mice at 7-8 weeks old (Jackson Laboratory Japan, Inc., Yokohama) received ASO or saline (10 µL/head) via ICV or IT injection. The repeated IT group received the same dose once daily for 4 consecutive days. Blood was collected 4 h after each dose. Modified Irwin assessments were conducted 1 h post-dose; in the repeated IT group, evaluations were performed after the first and last administrations. Tissues were harvested 14 days post-dose. All animal experiment protocols were approved by the IACUC of Shonan Health Innovation Park.



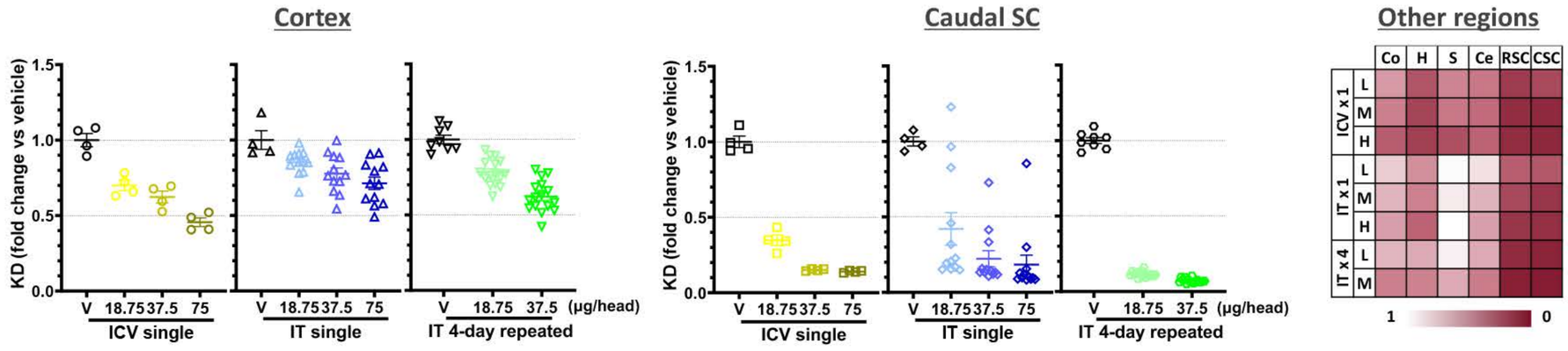
Quantitative RT-PCR

Mouse *Malat1* mRNA expression was quantified by quantitative RT-PCR using standard methods.
Mouse GAPDH was used as an internal control.

Measurement of ASO concentration

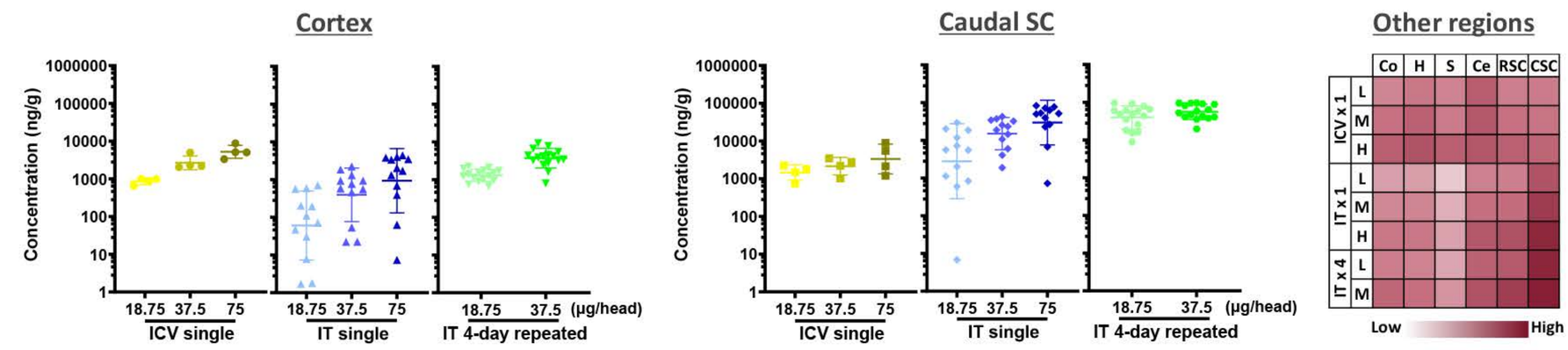
ASO concentration in plasma and tissue was measured by Hybridization ELISA.

Target Knockdown Across Dosing Routes



***Malat1* mRNA knockdown (KD) in CNS target regions 14 days post-dose following single ICV, single IT, or 4-day repeated IT dosing of MALAT1 ASO.**
Group sizes: ICV, N=4 (all groups); IT single, N=4 (vehicle) and 12 (ASO); IT repeated, N=8(vehicle) and 16 (ASO). KD in cortex and caudal spinal cord is shown as individual values with mean ± SEM; other regions are presented as a heatmap (0–1). Abbreviations: Co, cortex; H, hippocampus; S, striatum; Ce, cerebellum; RSC, rostral spinal cord; CSC, caudal spinal cord. Doses: L, 18.75 µg/head; M, 37.5 µg/head; H, 75 µg/head.

Exposure in CNS Target Regions



Exposure in CNS target regions 14 days post-dose following single ICV, single IT, or 4-day repeated IT dosing of MALAT1 ASO.
Data are from the same animals as in the KD panel above. Other regions: shown as a log-transformed heatmap (normalized to 0–100%)

Neurological Safety Assessment

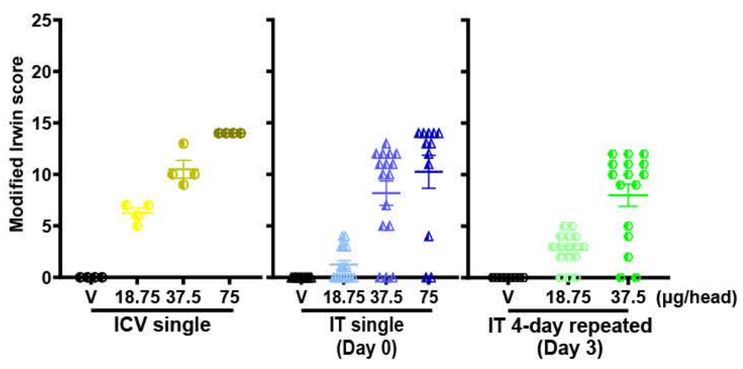
- ✓ Modified Irwin assessment, optimized for ASO evaluation
- ✓ Total score: 25 points based on the items below; death = 26 points

Categories and Scoring

Category	Score	Category	Score
Activity / Excitation		**Nerve / Muscle**	
Bizarre behavior	0, 1	Abnormal posture	0, 1, 2, 3
Locomotor activity	0, 1, 2, 3	Abnormal gait	0, 1, 2, 3
Tremors	0, 1	Grip strength	0, 1
Convulsions	0, 1, 2, 3	**Autonomic**	
Abnormal breathing	0, 1	Piloerection	0, 1
Sensory / Motor		Skin abnormalities	0, 1
Irritability	0, 1, 2, 3	Salivation	0, 1
Righting reflex	0, 1, 2, 3	**Death**	0, 26

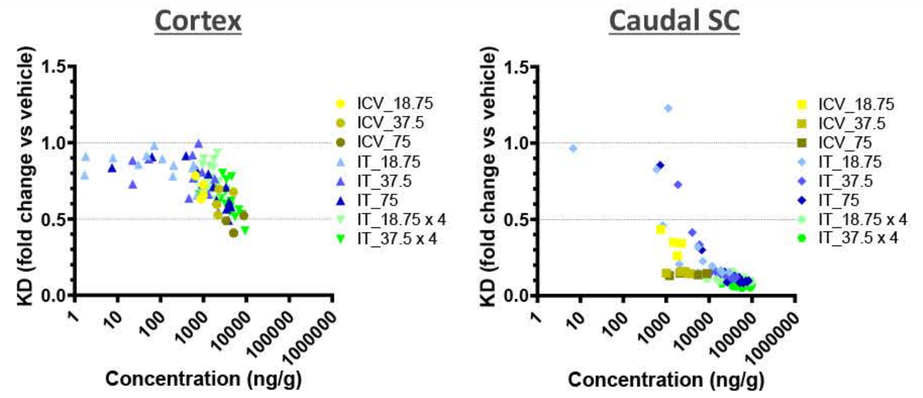
Ref. Nihon Yakurigaku Zasshi. 2021;156(3):171-177.

Modified Irwin score



Modified Irwin score at 1 h post-dose.
In the repeated IT group, assessments were performed 1 h after the first and last doses.

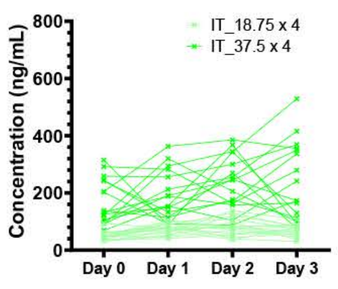
PK–PD Correlation



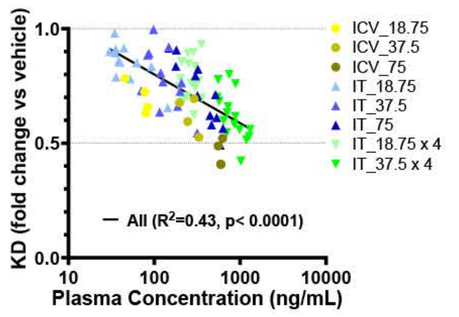
Correlation between tissue exposure and *Malat1* mRNA KD in cortex and caudal spinal cord at 14 days post-dose.
Data shown here were derived from the datasets presented above.

Plasma PK and Predictive PD

Plasma Concentration



Plasma Concentration–KD Relationship



Plasma PK concentration in the repeated IT group (left) and Predictive relationship between plasma concentration on the dosing day and cortical KD at 14 days post-dose (right).
For the repeated IT group, plasma concentrations represent the sum of values over the 4 dosing days.

Conflict of Interest Disclosure

The presenter has no conflicts of interest to disclose related to this presentation within the past three years.

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