** EBRAINS Data Descriptor**

*The HBP Data Descriptor follows closely the data descriptor template of the journal Scientific Data (ISSN 2052-4463, https://www.nature.com/sdata/). Note that sections marked with an asterisk are obligatory.*

**TITLE\***

*110 characters maximum, including spaces, no colons and parentheses*

*The title should be descriptive for the presented data. The use of acronyms and abbreviations should be avoided where possible.*

Intracerebroventricular and intrathecal delivery routes for ASOs targeting *Dmd* exon 51 in *mdx52* mice

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**ABSTRACT\***

*170 words maximum, no references*

*The abstract should concisely describe the study, the assay(s) performed, the resulting data, and their reuse potential, but should not make any claims regarding new scientific findings.*

Nucleic acid-based therapies have demonstrated great potential for the treatment of monogenetic diseases, including neurologic disorders. To date, regulatory approval has been received for a dozen antisense oligonucleotides (ASO), however these chemistries cannot readily cross the blood-brain-barrier when administered systemically. Therefore, an investigation of their potential effects within the central nervous system (CNS) requires local delivery. Here we studied the brain distribution and exon-skipping efficacy of two ASO chemistries, PMO and tcDNA, when delivered to the cerebrospinal fluid (CSF) of mice carrying a deletion in exon 52 of the dystrophin gene, a model of Duchenne muscular dystrophy (DMD). Following intracerebroventricular (ICV) delivery (unilateral, bilateral, bolus vs slow rate, repeated via cannula or very slow via osmotic pumps), ASO levels were quantified across brain regions and exon 51 skipping evaluated, revealing that tcDNA treatment invariably generates comparable or higher skipping relative to PMO, even when the PMO was administered at higher doses. We also performed intra-cisterna magna (ICM) delivery as an alternative route for CSF delivery and found a biased distribution of the ASOs towards posterior brain regions, including the cerebellum, hindbrain, and the cervical part of the spinal cord. Finally, we combined both ICV and ICM injection methods to assess the potential of an additive effect of this methodology in inducing efficient exon skipping across different brain regions.

**BACKGROUND & SUMMARY**

*700 words maximum, optional section*

*This section should provide a more detailed overview of the study, the assay(s) performed, and the resulting data as well as referencing previous work and related literature to put the study into context. This section should also briefly outline the broader goals that motivated collection of the data, as well as their potential reuse value.*

Antisense oligonucleotides (ASOs) hold tremendous therapeutic potential for many genetic diseases. ASOs are short, synthetic, single-stranded oligonucleotides that can bind to mRNA and non-coding RNAs to reduce, restore, or modify protein expression 1–3. Several ASOs have already reached market approval, in particular for the treatment of neuromuscular disorders like spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD). DMD is a neuromuscular disease with an incidence of 1:5000 boys, who carry mutations of the *DMD* gene that disrupt the open-reading frame of the dystrophin protein (Dp) in muscle, heart and brain 4,5. The ASOs used in DMD aim to restore the open reading frame to produce an internally deleted but still functional protein 6–8. Over the past decade, a number of ASOs have been developed to target exons flanking different relatively common groups of DMD mutations, leading to four such therapies being conditionally approved by the FDA. These ASOs require regular (weekly) systemic administration via intravenous route. However, none of these ASOs can address DMD brain comorbidities which have a significant impact on the patients’ quality of life. These include intellectual disability and neurobehavioural comorbidities, affecting between 30 and 50 % of individuals with DMD 9,10. The European *Brain Involvement in Dystrophinopathies* (BIND) consortium (<https://bindproject.eu/>) aims to investigate and characterise further the role of the various dystrophin isoforms in the CNS and evaluate the potential reversibility of the central deficits associated with the lack of dystrophin. In this context, we are using the exon52-deleted *mdx52* mouse model of DMD 11, as this mutation is located in a “hot spot” region that is frequently mutated in DMD patients 10,12,13. We previously showed that *mdx52* mice, lacking Dp427, Dp260 and Dp140 display enhanced anxiety and fearfulness, and impaired associative fear learning as compared to the original Dp427 deficient-*mdx* mouse model.14 The development of therapeutic approaches in this mouse model is thus of great interest, as it directly translates to patients’ condition. As part of our investigations, we first aimed to optimize the administration route to achieve high and widespread delivery of ASOs in the CNS of DMD mouse models.

Considering that systemic delivery of ASO requires high doses and results in very low efficacy in the CNS, mostly due to the inability of oligonucleotides to efficiently cross the blood–brain barrier (BBB), we focused on local delivery to the CNS. One of the most common delivery method to the CNS is administration to the cerebrospinal fluid (CSF) 15,16. CSF continuously flows in cerebral ventricles, the subarachnoid, cisternal spaces and the spinal canal. Its direct contact with the CNS makes it an ideal delivery route to achieve widespread distribution to the CNS 17, although different regions are targeted with different efficiency. CSF delivery can be achieved *via* injection into the cerebral ventricles, the lumbar intrathecal space, or the *cisterna magna*. The most commonly used delivery route to the CSF, in particular for rodents, is *via* intracerebroventricular (ICV) injection based on specific stereotaxic coordinates 18. On the other hand, intrathecal (IT) delivery *via* lumbar puncture is a convenient delivery strategy not only because it does not require a stereotaxic set up but also because of ease of access and minimal invasiveness 19–21. This method results in efficient drug distribution to the spinal cord in animal models and patients and is successfully used in the clinic for the administration of the ASO Nusinersen to SMA patients 22. However, preclinical data in animal models also suggest that lumbar intrathecal delivery results in limited distribution to the supratentorial brain structures 23 that are the target for DMD therapies. As an alternative to lumbar delivery, administration into the *cisterna magna* has been used in animal models to achieve delivery closer to brain structures. This method has resulted in widespread distribution to the cerebellum, hindbrain and spinal cord in mice 23.

In the present study, we aimed to optimize ASO efficacy in the CNS, to reach the highest possible exon skipping levels in brain regions known to express dystrophin such as the cerebellum, hippocampus, and cortex. We therefore evaluated different delivery methods, in particular ICV and ICM (intra-cisterna magna) using various injection regimens. Notably, we compared unilateral *vs* bilateral ICV injections, bolus *vs* slow rate of injection as well as repeated administration of ASO using a cannula and continuous administration using osmotic pumps. Finally, we studied the feasibility and resulting effects of combining both ICM and ICV delivery methods.

Considering that ASO biodistribution in the CNS may be affected by various factors such as ASO chemistry and charge, we used 2 different chemistries of ASO in this study: the Phosphorodiamidate Morpholino Oligomer (PMO) chemistry and the tricyclo-DNA (tcDNA) chemistry. PMOs have a neutral charge and are already approved by the FDA for the systemic treatment of DMD 8, while tcDNA is a charged, lipid-conjugated ASO that has previously shown therapeutic potential in mouse models of DMD 24,25. Overall, our detailed comparative study provides useful insights into the local delivery and associated efficacy of ASOs in the CNS of mouse models of DMD.

**METHODS\***

*This section should be used to describe any steps or procedures that produced the data, including full descriptions of the experimental design, data acquisition assays, and any computational processing (e.g., normalization, image feature extraction). Each method should be described under correspondingly named subheading. It is acceptable to cite previous descriptions of the methods under use, but the descriptions should be complete enough for others to understand and reproduce the methods and processing steps without reading the associated publications. There is no word limit to the length of this section.*

The methods used to produce the data have been described previously in 25,26:

Zarrouki, F., Relizani, K., Bizot, F., Tensorer, T., Garcia, L., Vaillend, C., & Goyenvalle, A. (2022). Partial Restoration of Brain Dystrophin and Behavioral Deficits by Exon Skipping in the Muscular Dystrophy X-Linked (mdx) Mouse. Annals of neurology, 92(2), 213–229. https://doi-org.proxy.insermbiblio.inist.fr/10.1002/ana.26409

Saoudi, A., Barberat, S., le Coz, O., Vacca, O., Doisy Caquant, M., Tensorer, T., Sliwinski, E., Garcia, L., Muntoni, F., Vaillend, C., & Goyenvalle, A. (2023). Partial restoration of brain dystrophin by tricyclo-DNA antisense oligonucleotides alleviates emotional deficits in mdx52 mice. Molecular therapy. Nucleic acids, 32, 173–188. <https://doi-org.proxy.insermbiblio.inist.fr/10.1016/j.omtn.2023.03.009>

**Experimental design study ICV**:

Adult (6-8-wks old) male *mdx52* mice received an intracerebroventricular (ICV) injection of 400 ug of tcDNA-ASO targeting exon 51 (group ICV tcDNA-Ex51, n=5, mouse ID: T01-T05) or saline control (group PBS, n=2, mouse ID: PBS01 – PBS02).

A second group of 6-wk-old male mdx52 mice received an ICV injection of 900 ug of PMO-ASO targeting exon 51 (group ICV PMO-Ex51, n=3, mouse ID: T01-T03) or saline control (group PBS, n=2, mouse ID: PBS03 – PBS04).

**Experimental design study repeated ICV & osmotic pumps**:

Adult (6-8-wks old) male *mdx52* mice were implanted with cannula and received 3 ICV injections of 400 ug of tcDNA-ASO targeting exon 51 (group Cannula tcDNA-Ex51, n=3, mouse ID: C01-C03), 5 ICV injections (group tcDNA-Ex51, n=3, mouse ID: CC01-CC03) or saline control (group PBS, n=2, mouse ID: PBS01 – PBS02).

A group of (6-8-wks old) male *mdx52* mice were implanted with cannula and received 3 ICV injections of 900 ug of tcDNA-ASO targeting exon 51 (group Cannula PMO-Ex51, n=3, mouse ID: C01-C03) or saline control (group PBS, n=2, mouse ID: PBS03– PBS04)

Adult (6-8-wks old) male *mdx52* mice were implanted with osmotic pumps and received 1mg of tcDNA-ASO targeting exon 51 (group Pump tcDNA-Ex51, n=3, mouse ID: P01-P03) or saline control (PBS, n=2, mouse ID: PBS05 – PBS06).

**Experimental design study ICM**:

Adult (6-8-wks old) male *mdx52* mice received an intra *cisterna magna* (ICM) injection of 400 ug of tcDNA-ASO targeting exon 51 (group ICM tcDNA-Ex51, n=4, mouse ID: T01-T04) or saline control (group PBS, n=2, mouse ID: PBS01 – PBS02).

A second group of 6wks old male mdx52 mice received an ICM injection of 900 ug of PMO-ASO targeting exon 51 (group ICM PMO-Ex51, n=3, mouse ID: T01-T03) or saline control (group PBS, n=2, mouse ID: PBS03 – PBS04).

**Experimental design study repeated ICM**:

Adult (6-8-wks old) male *mdx52* mice received 3 ICM injections of 400 ug of tcDNA-ASO targeting exon 51 (group Triple ICM tcDNA-Ex51, n=6, mouse ID: I01-I06) or saline control (group PBS, n=2, mouse ID: PBS01 – PBS02).

A group of (6-8-wks old) male *mdx52* mice received 3 ICM injections of 900 ug of tcDNA-ASO targeting exon 51 (group Triple ICM PMO treated, n=3, mouse ID: I01-I03) or saline control (group PBS, n=2, mouse ID: PBS03– PBS04)

**Experimental design study combined ICV and ICM**:

For the treatment combining ICM and ICM, a separate group of 6-week-old *mdx52* male mice underwent an ICM injection followed by an ICV of 400 ug of tcDNA-ASO targeting exon 51, (group Combined ICV+ICM tcDNA-Ex51, n=4, mouse ID T01-T04) or saline (group PBS, n=2, mouse ID: PBS01 – PBS02).

A group of (6-8-wks old) male *mdx52* mice received 3 ICM followed by an ICV injections of 900 ug of tcDNA-ASO targeting exon 51 (group Combined ICV+ICM PMO-Ex51, n=4, mouse ID: T01-T04) or saline control (group PBS, n=2, mouse ID: PBS03– PBS04).

7 weeks post-treatment, all mice were euthanized and brain and muscles samples were collected for further molecular analysis (% of Exon 51 skipping).

**TECHNICAL VALIDATION\***

*This section should present any procedure that is needed to support the technical quality of the data. It should justify the reliability of the presented data. This may include: experiments supporting or validating the data-collection procedure, statistical analyses of experiment errors and variation, phenotypic or genotypic assessments of biological samples, any procedure used to ensure reliable and unbiased data production, acclimatisation procedures to ensure the protection of personal data, etc.. This should not include: follow-up experiments aimed at testing/supporting an interpretation of the data, statistical hypotheses testing, or exploratory computational analyses like clustering and annotation enrichment.*

**USAGE NOTES\***

*This section should contain brief introductions to assist others with reuse of the presented data. This can include suggestions of software packages that are suitable for analyzing the presented data, or tips for further processing steps and for integrating or compare the presented data with other data.*

*Under a subsection called “SPATIAL ANCHORING”, information should be provided that facilitates the spatial anchoring of the presented data into the HBP interactive atlas viewers. If possible, brain image data and coordinates should be provided not only in native space, but in one of the following brain reference spaces: BigBrain template [v1], MNI Colin27 [v1], MNI ICBM 152 [2009c, nonlinear, asymmetric], Infant brain template [v4.0], Allen Mouse CCF [v2 or v3], WHS SD atlas template [v2.0]. Semantic links preferred to brain regions of one of the following atlases: BigBrain parcellation [v1], JuBrain probabilistic cytoarchitectonic atlas [v18], Infant brain atlas [v4.0], Allen Adult Mouse Brain Reference Atlas [v2 or v3], WHS SD atlas [v2.0].*

**SPATIAL ANCHORING:**

**DATA RECORDS\***

*This section should be used to explain the data presented in this descriptor and the repository where they are stored. This should include an overview of data files and formats, and potentially a short content description and file-internal data structure for each file type. Example of a mock-up repository overview:*

In the repository the data are stored in the following structure (incl. info on file content):

**repository-root/**

**data-descriptor.pdf** *[contains a short description of the dataset*

**sub\_info.tsv** *[contains information on the subjects*

**experimental-methods\_info.json** *[contains information on the applied experimental method]*

**sub-XXX/**

**sub-XXX\_slice-XXXX.tif** *[brain slice scan of subject XXX; index XXXX equals physical slice position in µm, anterior to posterior]*

**derived-data/**

**analysis-methods\_info.json** *[contains information on the applied analysis methods]*

**analysis-X/**

**analysis-X\_set-X.tsv** *[contains result data of analysis X]*

**code/**

**analysis-X.py** *[script that produces result data of analysis X]*

Information on used file formats and file-internal data structures:

**Tab-Separated Value format (tsv):** *labels in first row; data of same type in columns*

**JavaScript Object Notation (json):** *nested key-value pairs [cf. templates in xx]*

**Tagged Image Format File (tif):** *100µm thickness; 1x1 pixel dimension; unit in µm*

**Python Script (py):** *Python 3.0 script*

In the repository, the data are stored under an excel format.

The datasheet ‘ASO delivery optimization’ contains 6 sheets:

1- Summary of metadata

2- ICV skipping %

3- Repeated ICV skipping %

4- ICM skipping %

5- Repeated ICM skipping %

6- Combined ICM+ICV skipping %

In each sheet, the subject and the tissue samples are organized in lines.

**CODE AVAILABILITY\***

*This section should list all software / code (ready-made or custom-made) and their version used in the generation or processing of the presented data. This should include a statement indicating whether and how the software or code can be accessed, including any access restrictions. If relevant, provide also any specific variables or parameters used to run the software / code on the presented data.*

**Acknowledgements**

*This section should contain brief acknowledgements of non-author contributors. Anonymous referees and editors or effusive comments are not accepted. Grant or contribution numbers can be acknowledged.*

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**Author contributions**

*This section should state briefly on a separate line each author’s contribution to producing or maintaining the presented data as well as their role in publishing them.*

Conceptualization and methodology F.M. and A.G.; Analysis, A.S., C.F., C.V., T.G.; Investigation, A.G., A.S., C.F., C.V., F.M., J.M., T.G., V.K.; Resources, F.M. A.G. C.V. V.K., T.T.; L.G.; F.M; writing—original draft preparation A.S., A.G.; writing—review and editing A.G., A.S., C.F., C.V., F.M., J.M., T.G., V.K.; supervision, A.G, V.K., F.M.; funding acquisition, F.M.

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