REPORT

Leriglitazone halts disease progression in adult patients with early cerebral adrenoleukodystrophy

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Abstract

Cerebral adrenoleukodystrophy (CALD) is an X-linked rapidly progressive demyelinating disease leading to death usually within a few years. The standard of care is hematopoietic stem cell transplantation (HSCT), but many men are not eligible due to age, absence of a matched donor, or lesions of the corticospinal tracts (CST). Based on the ADVANCE study showing that leriglitazone decreases the occurrence of CALD, we treated 13 adult CALD patients (19-67 years of age) either not eligible to HSCT (n= 8) or awaiting HSCT (n= 5).

Patients were monitored every 3 months with standardized neurological scores, plasma biomarkers and brain MRI comprising lesion volumetrics and diffusion tensor imaging.

The disease stabilized clinically and radiologically in 10 patients with up to 2 years of follow-up. Five patients presented with gadolinium enhancing CST lesions that all turned gadolinium negative and, remarkably, regressed in four patients. Plasma neurofilament light chain levels stabilized in all 10 patients and correlated with lesion load. The two patients who continued to deteriorate were over 60 years of age with prominent cognitive impairment. One patient rapidly died from Covid19.

These results suggest that leriglitazone can arrest disease progression in adults with early-stage CALD and may be an alternative treatment to HSCT.

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Introduction

Adrenoleukodystrophy (ALD) is a rare X-linked white matter disease caused by mutations in the *ABCD1* gene. Pathogenic *ABCD1* variants lead to an impairment of peroxisomal beta-oxidation and accumulation of very long-chain fatty acids in plasma and tissues. Symptoms are linked to the dysfunction of organs that are highly dependent on fatty acid metabolism, such as the adrenal cortex, the spinal cord and the brain.[1]

All men carrying *ABCD1* pathogenic variants develop a myeloneuropathy (adrenomyeloneuropathy, AMN), which causes progressive spastic paraparesis, sensory ataxia and neurovegetative dysfunctions.[2] These patients are also at high risk of developing a rapidly progressive leukodystrophy (cerebral ALD, CALD) in which inflammatory demyelinating lesions lead to severe motor and cognitive deficit, bedridden condition, and death.[3] Approximately one third of boys and more than half of men may be affected by CALD,[4,5] but the individual risk of CALD conversion cannot be predicted by genotype.[6]

The diagnosis of CALD is currently based on brain MRI, which shows white matter lesions that are typically located in the corpus callosum, corticospinal tracts (CST) and cerebellum.[7] In children with CALD, these lesions have a typical peripheral enhancement after gadolinium injection which correlates with disease progression.[8] In adults with CALD, the MRI pattern can be more complex,[9] due to diffuse white matter signal abnormalities associated with AMN, and the variability of contrast enhancement. The Loes score reflects global disease severity,[10] and is used to guide therapeutic management of patients with CALD.[4] Diffusion tensor imaging (DTI) parameters, including fractional anisotropy (FA) and mean diffusivity (MD), are relevant *in vivo* myelin markers in humans.[11] In patients with AMN, they were sensitive markers to evaluate disease progression and therapeutic efficacy.[12–15] However, their relevance and applicability in CALD management needs to be further investigated.

Hematopoietic stem cell transplantation (HSCT) is the standard of care for CALD. It can halt neuroinflammation through the replacement of microglia,[9] even though its precise mechanism is not fully understood.[16] Its efficacy is strongly related to the precocity of diagnosis and treatment.[17] In men with myeloneuropathy, the identification of CALD conversion remains a

major challenge due to the non-specificity of clinical symptoms and low sensitivity of the Loes score at the early disease stages.[18] The latest study of HSCT in adult patients with CALD has reported poor outcomes in case of severe AMN (EDSS > 6), cognitive impairment and bilateral lesions of internal capsules.[19] Too often, the diagnosis of CALD is made when HSCT is no longer possible, with a mean survival ranging from 2 to 3 years.[5,20]

Leriglitazone is a brain-penetrant peroxisome proliferator-activated receptor gamma (PPARγ) full agonist. It regulates the expression of key genes that modulate redox status, mitochondrial function and biogenesis, and myelination. In addition, the compound decreases inflammation through downregulation of the NF-κB signaling pathway.[21] Leriglitazone can cross the intact blood brain barrier (BBB) and protect oligodendrocytes from death, and neurons from demyelination, thereby promoting remyelination.[21] It increases astrocyte and neuron survival, and decreases microglia activation.[21] Leriglitazone can reduce monocyte adhesion to activated endothelial cells of the BBB, a mechanism with a prominent role in the initiation of CALD and reduce underlying inflammation.[21] Leriglitazone decreased the levels of several pro-inflammatory cytokines in CSF and plasma and increased adiponectin concentrations, a biomarker for PPARγ engagement, in healthy volunteers and in patients.[21,22]

The ADVANCE study tested this molecule on 116 male patients with AMN in a randomised and placebo-controlled trial. It confirmed its safety and showed an effect on myelopathy through a reduction of body sway amplitudes, a stabilisation of walking aids and a slower decline of ambulation scores in early-stage patients. Further analyses showed that cerebral lesion progression occurred significantly more often in the placebo group, suggesting a preventive effect on the occurrence of CALD.[22] The slowing of cerebral lesion progression was supported by exploratory data on plasma biomarkers including Neurofilament light chain (NfL), Matrix metalloproteinase 9 (MMP-9) and inflammatory cytokines such as IL-1Ra, IL-18 and MIP-1β (CCL-4).[22]

Here, we report the safety and efficacy of leriglitazone in 13 adult patients with CALD, either not eligible to HSCT or awaiting HSCT, who benefitted from a compassionate use of the drug in France. Patients were thoroughly evaluated by standardized clinical scores, non-subjective neuroradiological metrics (lesion load and calibrated diffusion tensor analyses), and plasma biomarkers.

Materials and methods

Study conduct

Adult patients with CALD were prospectively enrolled between November 2021 and April 2023 at La Pitié-Salpêtrière University Hospital (Paris, France) and were treated with leriglitazone as part of an early access program from the French drug agency (ANSM) that allows for the use of innovative treatments with favorable safety profiles (ATU). The study was approved by Inserm IRB00003888, FWA00005831, opinion number 18-536 and written consent was obtained from all participants.

Leriglitazone was administered orally at a daily single dose of 150 mg.

Patients were assessed every 3 months with clinical examination including the Expanded Disability Status Scale (EDSS), the Adult Adrenoleukodystrophy Clinical Score (AACS), plasma samples and brain MRI. Cognitive evaluation was performed by a neuropsychologist at baseline and after 12 months of treatment. Adverse events were recorded during visits and monthly calls.

Participants

Patients were men aged from 19 to 67 years with i) genetically confirmed ALD, ii) progressive (i.e., increased lesion load on consecutive scans, with or without gadolinium enhancement) and clinically relevant CALD lesions on brain MRI, iii) either not eligible to HSCT (age > 55 years, lack of a suitable donor, inflammatory lesions of the CST), or awaiting HSCT. Bedridden patients were not treated.

MR Imaging Protocol

All scans were acquired on a 3.0 Tesla MRI scanner. The protocol included a 3D T_1-weighted (T_1-w) magnetization prepared rapid gradient echo (MPRAGE) sequence, a T_2-w fluid attenuated

inversion recovery (FLAIR) sequence, a diffusion-weighted sequence, and a T_1-w spin echo sequence 5 minutes after gadolinium injection.

Image processing for volumetric analysis

CALD lesions were manually segmented on FLAIR sequences by a neuroradiologist (MG). Lesions considered as AMN-related were not segmented, after a consensus review with two ALD experts (FM, DG). Total lesion load and CST lesion load (involving the posterior limb of internal capsules, the cerebral peduncles and the pons) were calculated longitudinally (Supplementary Methods). Patients were excluded from analysis in case of excessive motion artefacts.

Image processing for diffusion analysis

The diffusion weighted images were processed with brainQuant module (Supplementary Methods) from the brainTale-care platform (version 2.2.0). We calculated the mean values of FA and MD of total white-matter, and the regional values of MD (expressed in standard deviations from the mean of 165 healthy volunteers), from a 19 regions white-matter atlas developed by Mori *et al.*[23] The sequences were excluded from this analysis in case of excessive motion, high number of global signal dropouts, vibration artefacts or co-registration failure.

Blood biomarkers analysis

Disease activity was also assessed through plasma levels of neurofilament light chain (NfL, an axonal integrity marker),[24] matrix metalloproteinase-9 (MMP9), CCL4, IL-1 receptor agonist (IL-1Ra), IL-18, CXCL1, CXCL8 and CCL2 (Supplementary Methods). PPARγ target engagement was evaluated through plasma adiponectin.[22,25]

Statistical analysis

The associations between plasma biomarkers, lesion volume and diffusion markers were quantified using Spearman´s correlation coefficient and a graphical representation of this association was fitted by linear regression in GraphPad Prism 8.0.

Results

Baseline patients' characteristics

We enrolled 13 patients, including five patients awaiting HSCT (#03, #04, #08, #09, #12) and eight patients not eligible to HSCT. Ten patients had gadolinium-enhanced lesions, including five (#01, #06, #10, #12, #13) involving the CST (Table 1). One patient (#01) had a medical history of posterior fossa ependymoma treated by surgery and radiotherapy.

Mean age was 41.8 years old (median 41, range 19-67). Mean (median, SD) EDSS was 4.5/10 (4.5, 2.4), AACS 7.9/24 (7, 5.4), and brain MRI Loes score 4.9/34 (5, 3.2).

Two patients had no follow-up MRI (#05 discontinued treatment, #07 died). Two patients were excluded from volumetric analysis (#02, #13) and one from diffusion analyses (#13) due to motion artefacts.

Clinical follow-up

Clinical evaluations were stable in 10 patients (#01, #03, #04, #06, #08, #09, #10, #11, #12, #13) over a follow-up period of up to 24 months (Fig. 1A, 1B, Supplementary Table 1). Two patients exhibited mild improvement in proprioception (#03, #12) (Fig. 1A), and three patients slightly better cognitive performances (#06, #08, #12) (Supplementary Table 1). Patient #07 died 3 months after treatment initiation due a severe Covid-19 infection, patient #05 progressed to a bedridden state and discontinued treatment after 8 months, and patient #02 continued treatment despite a clinical deterioration predominantly in cognitive functions (Fig. 1A, 1B, Supplementary Table 1).

Leriglitazone was well tolerated in all patients with minimal weight gain in most patients and moderate leg oedema in only two patients. Patient #05 had an episode of pleurisy, considered unrelated to the drug and with favourable evolution despite the continuation of treatment.

For all five patients awaiting HSCT, transplant has been put on hold given the successful therapeutic outcome so far.

Conventional imaging and volumetrics

The Loes score was assessed longitudinally for up to 24 months in the 11 patients for whom follow-up MRI scans were available (Fig. 1C). It remained unchanged in nine patients that were clinically stable. It increased in patient #02 in parallel to cognitive deterioration, and in patient #10 despite clinical stability (Fig. 1A, 1B, Supplementary Table 1).

Volumetric analyses showed stable lesion load in seven patients (#03, #04, #06, #08, #09, #11, #12) but increased lesion load in patients #01 and #10 due to a marked increase in cerebellar white matter lesions. Notably, these two patients had the highest lesion load at baseline (Fig. 1E).

Among the five patients with CST lesions, decreased CST lesion load was measured by volumetric analysis in three patients (#01, #06, #12) (Fig. 1F) and on visual assessment for patient #13.

Contrast enhancements decreased in 6/10 patients, with complete disappearance of contrast in four patients (#06, #09, #12, #13) (Table 1, Fig. 2). All the CST lesions turned gadolinium-negative after 3 to 6 months (Table 1). For patients #01 and #10, the favorable outcome on CST contrasted with increased cerebellar lesions that were gadolinium-positive (Supplementary Fig. 1). Two patients (#03, #08) displayed very mild persistent enhancements (Table 1).

Diffusion tensor imaging

Diffusion markers were assessed longitudinally in 10 patients (Table 1). Global FA and MD values in deep white matter were completely stable in six patients (#03, #04, #06, #08, #11, #12). Instead, they clearly worsened for patient #02 in line with his clinical deterioration. Only moderate variations were observed in patients with progressive cerebellar lesions (#01, #10), but

the cerebellum is not analyzed on the white matter atlas. For all patients with both a favorable clinical and radiological course, regional MD values tended to normalize over time (Fig. 3A-C). Instead, patient #02 displayed increased MD values in regions where lesions increased in size (Fig. 3D).

Plasma biomarkers

Biomarkers were measured longitudinally in all patients except #05 and #07 who discontinued treatment or died, respectively. Leriglitazone increased adiponectin concentrations at all time points tested (Supplementary Fig. 2A). NfL levels remained stable during longitudinal follow-up (from 3 to 24 months) for patients #03, #04, #06, #08, #09, #11 and #12 (Fig. 1D). Four patients (#01, #02, #10 and #13) had markedly elevated NfL at baseline compared to the other CALD patients, but levels stabilized in three patients (#01, #10, #13) with treatment whereas it kept increasing in patient #02, consistent with his clinical evolution (Fig. 1D). Supplementary Fig. 2B shows the detailed evolution of the other biomarkers.

Linear regression analyses

There was a statistically significant dependence of plasma NfL on FA ($r= -0.691$, $p< 0.001$), MD ($r= 0.515$, $p< 0.001$) and total lesion volume ($r= 0.611$, $p< 0.001$) (Supplementary Fig. 3A, 3B and 3E). Likewise, more advanced CALD patients (#01, #02, #10) presented both higher NfL levels (Fig. 1D), higher lesion load (Fig. 1E), and greater alterations of DTI metrics (Table 1). There was also a strong dependence of the total lesion volume on FA ($r= -0.773$, $p< 0.001$) and MD ($r= 0.780$, $p< 0.001$) (Supplementary Fig. 3C and 3D). Furthermore, NfL levels correlated with levels of CCL4 ($r= 0.434$, $p= 0.001$) (Supplementary Fig. 3F), a chemokine elevated in adult CALD patients.[25]

Discussion

This cohort study suggests that leriglitazone can halt neuroinflammation and disease progression on adult patients with CALD at early disease stages, as evidenced by clinical, radiological, and biological stability for over one year in most patients.

Out of ten patients whose disease stabilized, five patients exhibited mild clinical improvement with better proprioception, consistent with the possible benefit of leriglitazone reported on body sway in AMN patients,[22] or better cognitive subtests. These patients had low lesion load and low plasma NfL levels at baseline. This suggests a greater therapeutic impact at an earlier stage of CALD, in line with observations from HSCT.[17] Notably, the CST lesions regressed in 4/5 patients, with complete resolution of CST contrast enhancements. This could possibly reflect a greater action of leriglitazone on the motor pathways, which is of particular importance as CST inflammatory lesions are of poor prognostic for HSCT.[19]

The two patients (#01, #10) whose cerebellar lesion load increased despite stable motor and cognitive functions over one and 2 years of treatment respectively, had more extensive demyelinated lesions at baseline, including in the cerebellar white matter. We hypothesized that the medical history of posterior fossa ependymoma in patient #01 could induce a residual fragility in his cerebellum tissue, with a specific vulnerability to neuroinflammation and demyelination. In addition, both patients suffer from chronic alcohol addiction, which may further contribute to cerebellar metabolic vulnerability. Notably, cerebellar lesions decreased at M12 in patient #10 after complete alcohol withdrawal.

Besides the CALD patient who rapidly died from a severe Covid-19 infection, the two patients (#02, #05) with continued clinical and radiological deterioration were over 60 years of age and had prominent cognitive dysfunction at baseline. This is consistent with studies that have identified old age and altered cognition as risk factors of poor outcomes after HSCT.[19,26]

Global white matter diffusion metrics were strongly correlated with total lesion load and plasma NfL levels. Furthermore, changes in regional MD values mirrored volumetric evolution of lesions. A limitation though is the current restriction of diffusion metrics to the regions of a white-matter atlas that does not yet include the cerebellum. Nonetheless, disease progression in

two patients with cerebellar involvement (#01, #10) was associated with moderate changes of diffusion metrics, consistent with clinical stability in both patients.

The diffusion sequence has multiple advantages, including its accessibility on all MRI-scans and rapid acquisition time. The brainQuant pipeline from brainTale-care provides an automatic post-processing and a relative simplicity of interpretation, which makes it theoretically accessible to all imaging centres.

Besides its small sample size, the main limitation of this study is the absence of a placebo-controlled group. However, while we do not have a reference population that reflects the natural history of CALD lesions in adult patients, the evolution of most of our patients is very similar to what is observed in adult patients with early-stage CALD treated with HSCT,[19] especially the resolution of contrast enhancement and the stabilization of white matter lesion load.

Another limitation is that volumetric analysis of CALD lesions has intrinsic limited reproducibility. Indeed, in adults, CALD lesions are intertwined with those of AMN and may present blurred borders, particularly complex to segment reproducibly over time. Reproducibility errors were kept to a minimum using the same MRI-scan for each patient, the same reader for segmentations and the use of co-registration.

Leriglitazone was well tolerated in all patients. In the ADVANCE study, leriglitazone significantly decreased the occurrence of CALD in AMN patients. In this case-series, leriglitazone was associated with clinical stability and improved brain MRI patterns in early-stage CALD patients, over one year or more of treatment for most patients, similarly to what can be observed with HSCT. Therefore, leriglitazone may represent an alternative therapeutic approach in patients who are not eligible to transplant, or even after failed transplant. Larger confirmatory studies with leriglitazone in adult patients with CALD are nevertheless needed such as the ongoing US randomized controlled trial (NCT05819866). Leriglitazone may also be a new treatment for CALD patients with small lesions – like here patients #03, #04, #08, #09 and #12 with Loes score ≤ 5 – who may no longer need to undergo HSCT. However, while recurrence of CALD has not been observed when HSCT is successful, it has yet to be determined whether leriglitazone alone can permanently halt CALD.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Competing interests

Isabelle Weinhofer, Bernardo Blanco, Magali Barbier, Camille Huiban, Boris Chaumette and Bertrand Pichon report no competing interests. Marianne Golse, Elise Yazbeck and Johannes Berger serve as consultants for Minoryx therapeutics. Ali Fatemi serves as site principal investigator for a clinical trial sponsored by Minoryx therapeutics. Silvia Pascual is employee of, has patents with, and has stock options in Minoryx Therapeutics. Marc Martinell is co-founder and employee of, has patents with, and has stock options and shares in Minoryx Therapeutics. Vincent Perlbarg is co-founder and employee of, has patent with, and has stock options in BrainTale. BrainTale has received funding from Minoryx. Damien Galanaud is co-founder and has patent with BrainTale and serves as a consultant for Minoryx therapeutics. Fanny Mochel serves as a consultant and as site principal investigator for a clinical trial sponsored by Minoryx therapeutics.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Evolution of EDSS, AACS, Loes score, NfL, and white matter lesion load over time. (A) EDSS remained stable in most patients and slightly decreased in patients #03 and #12 with improved proprioception. Instead, it increased in patients #02 and #05. (B) AACS was unchanged in most patients but increased in patients #02 and #05. Patient #01displayed mild changes at M18 that stabilized at M24. (C) Loes score remained stable, except in patient #02, who deteriorated clinically, and in patient #10. It remained relatively stable in patient #01 despite extending white matter cerebellar lesions (counted as 1 regardless). (D) Higher NfL values were observed in patients with greater lesion loads. Most patients had stable NfL values over time. Patient #01 NfL values increased at M6 and M12, consistent with increased cerebellar lesion load, but decreased at M18 and M24 preceding clinical and radiological stabilisation. Patient #02 showed a continuous increase of NfL, in line with his clinical deterioration. Patient #10 displayed overall stable NfL values despite increased lesion load but consistent with clinical stability. (E) Total lesion load remained globally stable in most patients, whereas patients #01 and #10 showed a continuous increase of lesion load due to a progression of cerebellar white matter lesions. (F) CST lesion load continuously decreased in patient #01, slightly decreased in patients #06 and #12, and stabilized in patient #10.

Figure 2 Evolution of CST lesion load and gadolinium enhancements on brain MRI in two CALD adult patients. FLAIR sequences are shown in the upper rows and T1-w sequences after gadolinium injection are shown in the lower rows. (A) Patient #01 presented with bilateral lesions of his cerebral peduncles at baseline (arrows) with bilateral nodular enhancements (arrowheads). These lesions decreased in size and contrast enhancement disappeared from M6 onwards. (B) Patient #06 presented with bilateral lesions of his internal capsules at baseline (arrows) with bilateral enhancements (arrowheads). These enhancements decreased at M6 and disappeared from M12 onwards, with a lesion volume that appeared minimal at M24.

Figure 3 Evolution of the regional MD values (in standard deviations) in different regions of the deep white-matter atlas. (A) Patient #01 presented increased MD values between M0 and M6, that went back close to baseline values at M12 and increased again at M18. The transient and diffuse increase of MD values at M6 preceded an increase of the cerebellar lesion load at M12. Values at M24 were not available due to motion artifacts. (B, C) Patient #04 (B) and patients #06 (C) showed slightly elevated MD values at baseline, which decreased close to normal values at M6 and back to baseline values at M24. (D) Patient #02 showed increased MD values in all regions from M6 onwards, in line with clinical and radiological deterioration.

Table 1 Clinical and radiological evolution of CALD adult patients treated with leriglitazone.

	#01	#02	#03	#04	#05	#06	#07	#08	#09	#10	#11	#12	#13
Follow-up (m)	24	18	24	24	8	24	< 3	18	18	12	12	12	3
Age (y)	38	61	41	46	67	44	48	31	51	37	25	19	35
CST (FLAIR)	+ / +	+ / +	− / −	− / −	+ / NA[a]	+ / +	− / NA[b]	− / −	− / −	+ / +	− / −	+ / +	+ / +
CST (Gad)	3 / 0	0 / 0	0 / 0	0 / 0	0 / NA[a]	2 / 0	0 / NA[b]	0 / 0	0 / 0	1 / 0	0 / 0	1 / 0	1 / 0
Cerb (FLAIR)	+ / +	− / +	− / −	− / −	− / NA[a]	− / −	+ / NA[b]	+ / +	− / −	+ / +	− / −	− / −	+ / +
Cerb (Gad)	0 / 2	0 / 0	0 / 0	0 / 0	0 / NA[a]	0 / 0	2 / NA[b]	0 / 0	0 / 0	1 / 2	0 / 0	0 / 0	2 / 0
Other (Gad)	0 / 0	0 / 1	1 / 1	0 / 0	1 / NA[a]	0 / 0	1 / NA[b]	2 / 2	1 / 0	2 / 1	0 / 0	0 / 0	0 / 0
Global FA	−11% / −14%	−14% / −28%	−2% / −1%	−7% / −6%	−35% / NA[a]	−8% / −9%	−15% / NA[b]	−4% / −5%	−2% / −2%	−16% / −22%	−11% / −14%	−6% / −6%	NA[b]
Global MD	+12% / +17%	+10% / +22%	+1% / −3%	+8% / +7%	+46% / NA[a]	+8% / +7%	+9% / NA[b]	+6% / +7%	+3% / +6.5%	+11% / +21%	+15% / +16%	+7% / +9%	NA[b]

Cells containing two values show initial (left) and latest (right) scores. Gadolinium enhancement (Gad) is rated according to the Gadolinium Intensity Score,[28] which compares the contrast uptake intensity to the choroid plexus :0 = no enhancement, 1 = hypo-intense, 2 = iso-intense, 3 = hyper-intense.

[a]Tibial fracture introduced bias in clinical assessments.

[b]Important motion artefacts did not allow to perform diffusion analyses.

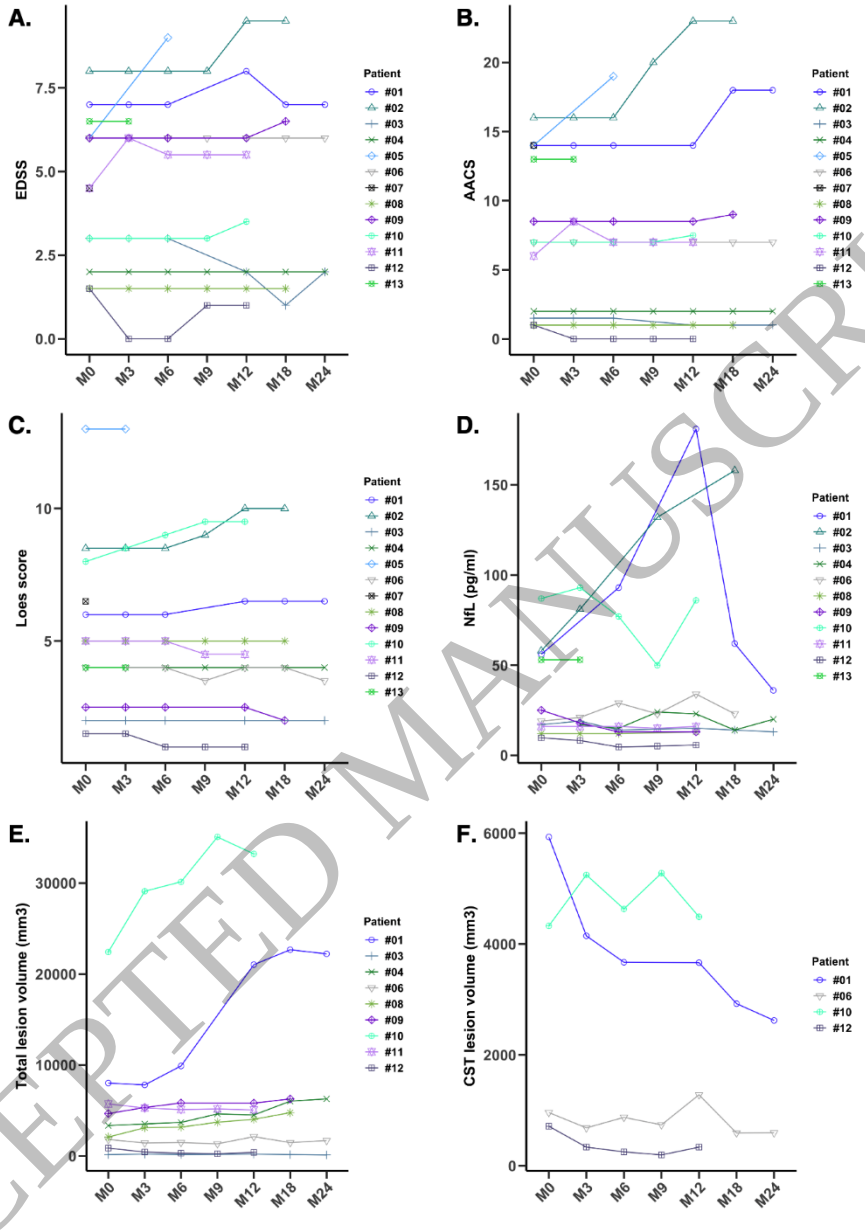

Figure 1
128x176 mm (DPI)

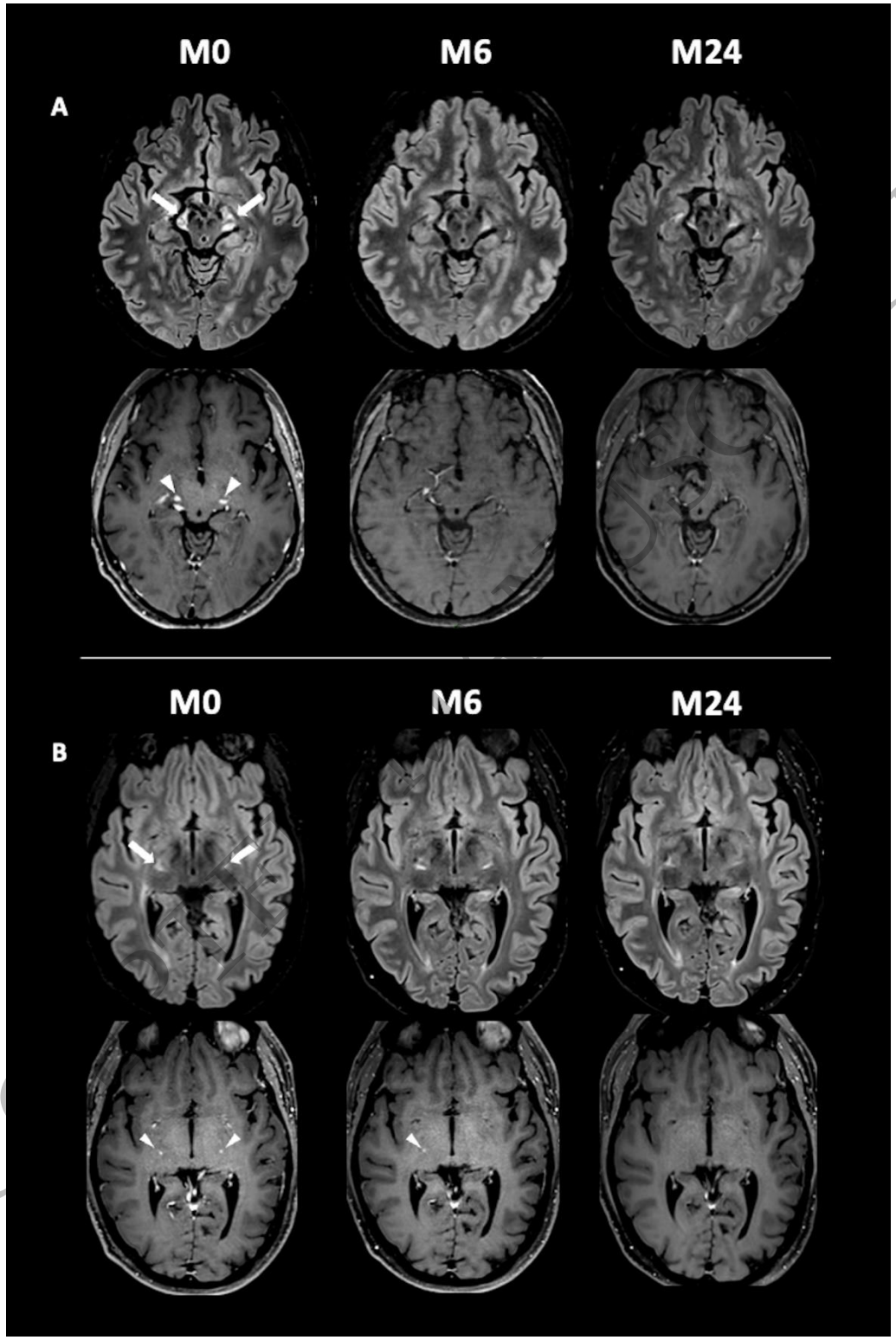

Figure 2
135x204 mm (DPI)

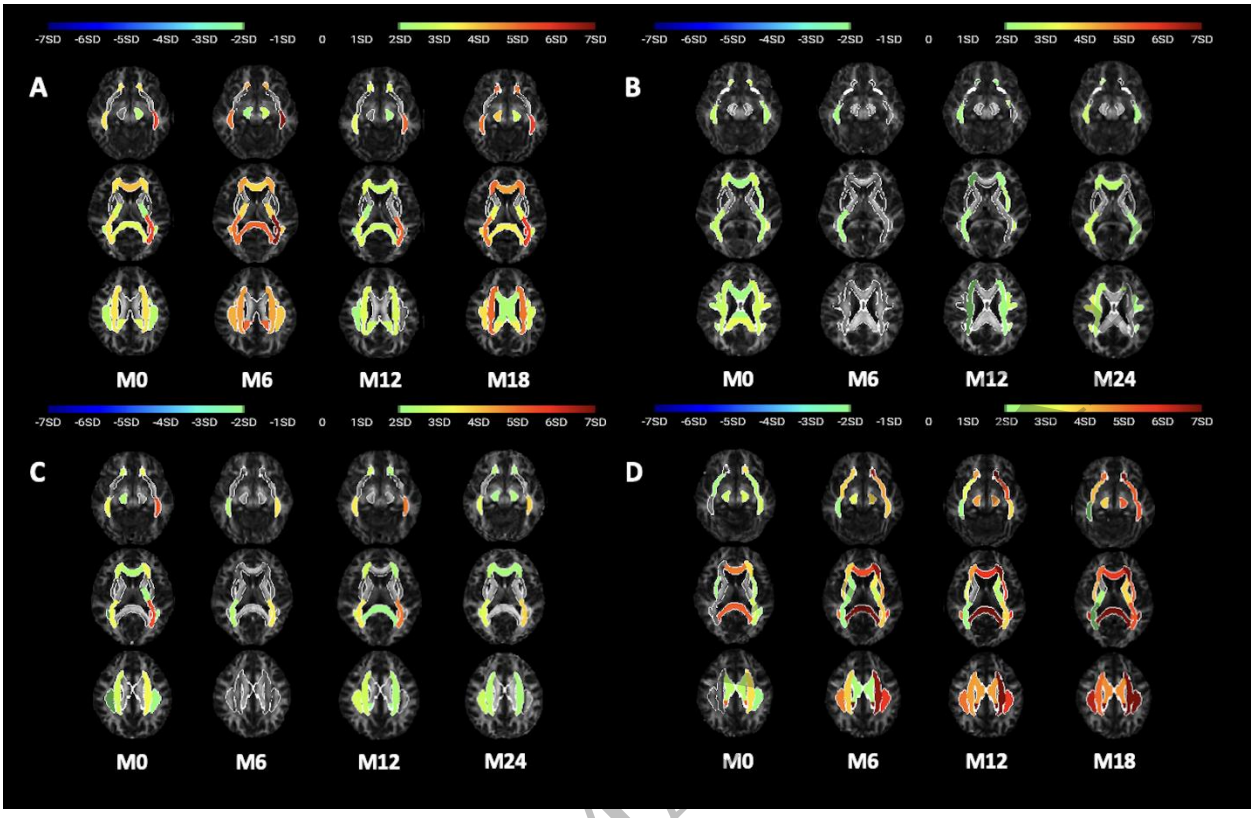

Figure 3
250x161 mm (DPI)