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Pharmacometabolomics applied to low-dose interleukin-2 treatment in amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron disease. The immunosuppressive functions of regulatory T lymphocytes (Tregs) are impaired in ALS, and correlate to disease progression. The phase 2a IMODALS trial reported an

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increase in Treg number in ALS patients following the administration of low-dose (ld) interleukin-2 (IL-2). We propose a pharmacometabolomics approach to decipher metabolic modifications occurring in patients treated with ld-IL-2 and its relationship with Treg response. Blood metabolomic profiles were determined on days D1, D64, and D85 from patients receiving 2 MIU of IL-2 ($n = 12$) and patients receiving a placebo ($n = 12$). We discriminated the three time points for the treatment group (average error rate of 42%). Among the important metabolites, kynurenine increased between D1 and D64, followed by a reduction at D85. The percentage increase of Treg number from D1 to D64, as predicted by the metabolome at D1, was highly correlated with the observed value. This study provided a proof of concept for metabolic characterization of the effect of ld-IL-2 in ALS. These data could present advances toward a personalized medicine approach and present pharmacometabolomics as a key tool to complement genomic and transcriptional data for drug characterization, leading to systems pharmacology.

KEYWORDS

amyotrophic lateral sclerosis, interleukin-2, kynurenine pathway, metabolomics, pharmacometabolomics

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and ultimately fatal motor neuron disease.¹ Drug discovery for this disease has been characterized by successive failures in clinical trials.^{2,3} Multiple pathological mechanisms have been identified in ALS, including the aggregation and accumulation of ubiquitinated protein inclusions in motor neurons, alterations in mRNA processing, glutamate-mediated excitotoxicity, oxidative stress, mitochondrial dysfunction, and neuroinflammation.^{1,4–8}

Regulatory T-lymphocytes (Tregs) are CD4⁺CD25⁺FoxP3⁺ cells that may act as negative regulators of inflammation. For example, they may play this key role by inhibiting effector cell activation.⁹ Treg immunosuppressive functions were found to be impaired in ALS patients, and a reduction in their levels was correlated to disease progression.^{8,10–13} Interleukin-2 (IL-2) is crucial for the maintenance and function of Tregs.¹⁴

IL-2 therapy has been shown to be capable of restoring Treg immunosuppressive functions *in vitro*.¹⁵ Based on the safety and the protective role of Tregs in ALS patients,¹⁶ the administration of IL-2 has been proposed as a new therapeutic strategy. The pharmacodynamics and safety of low-dose (ld) IL-2 was reported in the IMODALS study (NCT02059759)¹⁷ and showed a significant increase in Treg number with no serious adverse events. This phase 2a study was not designed to ascertain the efficacy of ld-IL-2 (1 or 2 MIU) on disease progression. However, we suspect a beneficial molecular effect associated with the changes in Treg number that merit further exploration. To achieve personalized medicine, the known heterogeneity of phenotypic characteristics and drug response of ALS patients requires an adaptation of the treatment strategy from initiation of treatment administration

to real-time adaptation. Relying solely on clinical characteristics for subgroup analysis to determine specific treatment protocols reveals insufficient sensitivity and robustness. Similarly, conventional methods to predict prognosis in treated patients are disappointing and probably inadequate. Interestingly, Giovannelli et al. attempted to answer the same question in a subgroup analysis in the same IMODALS cohort by performing microarray gene expression profiling. They identified transcripts associated with lipid metabolism that were linked to drug response.¹⁸

Pharmacometabolomics studies use metabolomic profiles to provide insight into the response to drug treatment. This approach can complement transcriptional studies by providing a deeper understanding of biochemical parameters associated with drug administration, which would assist in the characterization of a drug response and even disease heterogeneity.^{19,20}

Here, we describe a pilot study applying a pharmacometabolomics approach to plasma samples from the IMODALS cohort to provide new insight and contribute to efforts toward personalized medicine in neurology. To our knowledge, this study is the second pharmacometabolomics study performed in ALS patients.²¹ Our primary objective was to characterize the metabolic profile of patients treated with ld-IL-2 and its relationship with the changes in Tregs. The second objective was to explore the ability of baseline metabolomic profiles to predict Treg increase after treatment.

MATERIALS AND METHODS

Population

The study was performed using samples collected during the randomized (1:1:1), placebo-controlled, double-blind, parallel group trial

evaluating the safety and efficacy of Id-IL-2 in 36 ALS patients (NCT02059759). In this study, two doses of Id-IL-2 (1 and 2 MIU IL-2 per day) were tested and compared with placebo with an overall follow-up of 6 months and multiple regular blood collections performed over a 3-month period. According to previously published studies on this cohort, particularly the one reporting on Tregs, we decided to focus on (i) two groups of patients (the treatment group that received 2 MIU of IL-2, and the placebo group that received an injection of a 5% glucose solution) as 2 MIU of IL-2 was associated with more pronounced immune regulation and (ii) three time points for which blood samples were available: baseline (D1), 3 days after the last treatment cycle (D64), and 24 days after the last treatment (D85). All details concerning participants have previously been described in publications that reported the results of this clinical trial.^{17,18} The study protocol was submitted by the sponsor (Centre Hospitalier Universitaire de Nîmes) and approved by an independent ethics committee (Comité de Protection des Personnes Sud Méditerranée III; reference number: 2014.09.01-ter).

The following demographic and clinical data were documented: age of onset of first symptoms, site of symptom onset, disease duration from onset of symptoms to the first visit, sex, ALS revised Functional Rating Scale score, slow vital capacity, and body mass index (BMI). The clinical immunophenotyping that provided results of Tregs (defined as CD4⁺CD25⁺CD127^{low/-}FoxP3⁺ cell population) has been described in a previously published study.¹⁷ The increase in Treg number between D1 and D64 was calculated as follows: (Tregs at D64) – (Tregs at D1)/Tregs at D1.

Metabolomics analysis

Blood samples were collected at D1, D64, and D85 from all patients. After centrifugation at 3000×g for 10 min at room temperature, plasma samples were stored at –80°C until analysis. To explore the metabolomic profile, we used a targeted, quantitative approach via the AbsolutIDQ p180 kit (Biocrates) using flow injection analysis (FIA) and high-performance liquid chromatography (HPLC) mass spectrometry. This kit was chosen as it covers relevant pathways in our research fields. Importantly, it is a quantitative approach that may help in the specific routine for monitoring the relevant metabolites in the event of high relevance. It also uses a limited volume of samples with a high interlaboratory reproducibility, and its successful application has been consistently reported in the literature. This strategy allows for the quantification of 188 metabolites²²; 146 hydrophobic molecules via FIA and 42 polar metabolites via HPLC. The Biocrates Kit is based on the use of isotope-labeled internal standards for calibration curves and quality controls (QCs). Plasma samples were loaded onto filter paper and dried in a stream of nitrogen. Chemical derivatization was performed with a solution of 5% phenyl-isothiocyanate. Dried residues were extracted with methanol containing 5 mM ammonium acetate. The analysis was performed on a QTRAP 5500 System (AB Sciex) with an FIA method or coupled to HPLC using a 5 μm Ascentis Express C18

(4.6 × 250 mm) column. The MetIDQ software (Biocrates) was used to calculate concentrations of individual metabolites. Calibration curves and QCs were used to validate the analysis batch.

Statistical analysis

Baseline characteristics between the placebo and 2 MIU groups of patients were compared using Wilcoxon or chi-square tests.

First, metabolomics profiles were compared between the two groups at each visit. Variations between D1, D64, and D85 were then analyzed within each group. Since Treg analyses had previously revealed a high discrimination between groups at D64, we evaluated the relationship between Treg number and metabolite levels at D1 and their variation at D64 in both groups. Finally, we performed an analysis to estimate the ability of the metabolomic profile at D1 to predict the percent Treg number increase at D64 within the treatment group.

Given the limited number of patients, univariate analyses were based on nonparametric Wilcoxon and Spearman rank tests to evaluate metabolite differences between qualitative (treatment group) or quantitative (Treg number) variables, respectively. Friedman or Wilcoxon signed-rank tests were performed to compare quantitative values between three or two visits, respectively. *p*-Values for multiple comparisons were adjusted using the false discovery rate (FDR) method but due to the limited number of patients, and raw *p*-values were also considered to discuss trends.

For multivariate exploration, an unsupervised analysis via principal component analysis (PCA) was performed prior to the use of a supervised machine learning approach: sparse partial least-squares discriminant analysis (sPLS-DA) for a qualitative predicted variable or partial least squares regression (PLS) for a quantitative predicted variable. The MixOmics package was used to perform PLS and sPLS-DA analyses. A paired sPLS-DA was used to evaluate metabolomic changes between visits. The sPLS-DA models were tuned to select the optimal number of components and features. Concerning PLS, only those features with a variable importance in projection (VIP) value >0.8 were retained to build the final model. The quality of the models built was assessed by prediction accuracy following cross-validation (CV), and the significance was assessed by permutation tests (100 permutations using the function `MVA.test` of the `RVAideMemoire` package). We then used this fitted model to predict the percent Treg number increase at D64. The predicted values were compared to the observed values using linear regression. We chose these methods as they are particularly relevant when the number of observations is lower than that of the explanatory variables, which was the case here, and when the latter are correlated. To the best of our ability, we also limited the number of variables used in our models to avoid overfitting.

All analyses were performed using R studio software (version 2022.02.3). A *p*-value <0.05 was considered significant. Pathway analysis was conducted using the MetaboAnalyst software (<https://www.metaboanalyst.ca/>).

TABLE 1 Baseline characteristics of included patients.

	Placebo (N=12)	2 MIU (N=12)	Raw <i>p</i> -value
Sex			1
Female	3 (25%)	3 (25%)	
Male	9 (75%)	9 (75%)	
Age of onset (years)			0.86
Mean \pm SD	56.5 \pm 9.6	57.7 \pm 12.9	
Median (interquartile)	56.20 (48.78–63.85)	61.25 (46.57–64.75)	
ALSFRS-r			0.86
Mean \pm SD	38.83 \pm 3.35	37.67 \pm 5.25	
Median (interquartile)	38.5 (36.5–41.0)	39.0 (36.0–40.5)	
BMI (kg/m²)			0.18
Mean \pm SD	26.80 \pm 5.60	24.39 \pm 1.71	
Median (interquartile)	25.10 (24.05–27.27)	24.35 (22.95–26.02)	
Disease duration (year)			0.40
Mean \pm SD	2.21 \pm 1.44	1.96 \pm 1.44	
Median (interquartile)	1.75 (1.30–3.20)	1.45 (1.10–2.12)	
Treg number (cells/μL)			0.21
Mean \pm SD	52.17 \pm 17.59	66.04 \pm 29.65	
Median (interquartile)	48.25 (37.25–64.88)	64 (45.75–87.00)	

Abbreviations: ALSFRS-r, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; BMI, body mass index.

RESULTS

Population

The 12 patients receiving two MUI of IL-2 and the 12 control subjects had similar baseline characteristics (sex, age at onset, BMI, and site of onset; summarized in Table 1), as previously described¹⁷. Briefly, the mean (\pm SD) age was 57.7 \pm 12.9 years old in the treatment group compared to 56.5 \pm 9.6 years old in the placebo group. Treg number (cells/ μ L) was the same at baseline D1 (66.0 \pm 29.7) in the treatment group as in the placebo group (52.2 \pm 17.6) (Figure 1A). The increase in Treg number from D1 to D64 was significant in the treatment group (mean \pm SD), with an increase of 265 \pm 133% ($z = -3.49$, $p < 0.001$). The percent decrease in Treg number between D64 and D85 was significant in the treatment group (mean \pm SD), with an decrease of 60 \pm 19% ($z = -3.49$, $p < 0.001$) but not in the placebo group. To summarize, the change in Treg cell number over 85 days is represented in Figure 1B, and these findings were used for multivariate models.

Distinct change in metabolomic profiles over time between groups

A total of 181 metabolites (listed in Table S1) were analyzed in the blood of the 24 subjects, with pathways involving mainly those of amino acids and lipid metabolism.

Following univariate analysis (Wilcoxon test including FDR and *p*-values) at baseline, we did not find any significant difference between metabolites across the two groups. Metabolomic profiles showed no differences between the two groups as demonstrated by the PCA plot (Figure S1). Moreover, the sPLS-DA model showed poor performance (average error rate of 50%), and the permutation test was not significant.

At D64, phosphatidylcholine (PC) aa C32:3, C12:1, lysoPC a C26:1, PC ae 42:0, kynurenine, and PC aa C32:2 differed significantly between the two groups via raw *p*-values. Significant differences were not found with FDR correction. The model's ability to distinguish the two groups was not satisfactory (average error rate of 42%), although the permutation test was significant. Metabolites retained after adjusting the model were PC aa C32:3, lysoPC a C26:1, PC ae 42:0, PC aa C32:2, PC aa C34:4, PC aa C36:4, and kynurenine. Details about fold change and VIP values are given in Table S2. At D85, the model remained significant but with a nonsatisfactory performance (average error rate of 62.5%).

We then explored the metabolomic changes at the different time points within each group. Contrary to the findings for the placebo group, the paired sPLS-DA model was able to discriminate the three time points within the treatment group. This can be seen in the PCA plot with a correct separation of the metabolomes at the different time points (Figure 2A). The model was significant with modest accuracy (average error rate of 42%). The metabolites included in the model and their VIP values within each component are listed in Table S3. Among these metabolites, kynurenine had the highest VIP value in each component and the smallest *p*-value in univariate analysis

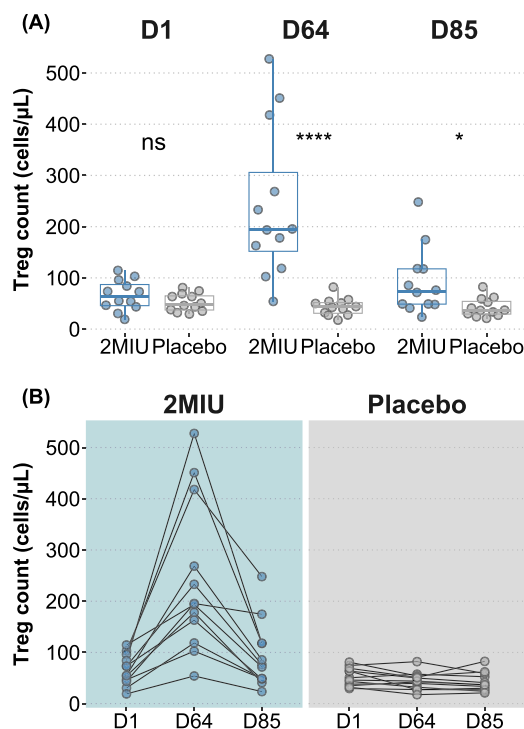


FIGURE 1 Effect of IL-2 treatment on Treg number. (A) Box plots representing the number of Tregs (cells/ μL) in the treatment group (blue) and the placebo group (gray box) at D1, D64, and D85. (B) Change in Treg number (cells/ μL) over time and between the treatment group (blue) and the placebo group (gray). Black lines connect the data points of a single patient. ns, $p > 0.05$; * $p < 0.05$; **** $p < 0.0001$.

($\chi^2 = 13.5$, raw p -value = 0.001, FDR-adjusted p -value = 0.13). Its level increased between D1 and D64 in almost all treatment group patients, followed by a reduction between D64 and D85 (Figure 2B).

Based on the metabolome response between D1 and D64 in the treatment group, a Wilcoxon signed rank test revealed significant changes in 16 metabolites (raw p -value < 0.05) but not after FDR correction. Among these, 12 corresponded to significant decreases in PCs (Table S4). Kynurenine and histidine increased and decreased significantly (raw p -value ≤ 0.001 ; FDR-adjusted p -value < 0.1), respectively. D1 and D64 were separated in the paired PCA (Figure 2C). The paired PLS-DA model was significant with good accuracy (overall error rate of 17%), with three metabolites retained to build the final model (kynurenine, PC aa C36:3, and PC aa C36:6).

Relationship between metabolomic profile and Treg response throughout treatment

With regard to the entire population, univariate analysis revealed that no metabolites were correlated with Treg number at baseline after FDR correction; however, five had a raw p -value < 0.05 (Spearman test [dfs = 22]: C14:2-OH, C14:2, PC aa C40:2, SM C26:1, and C5-DC (C6-OH)). The permutation test of the PLS model built to explain the

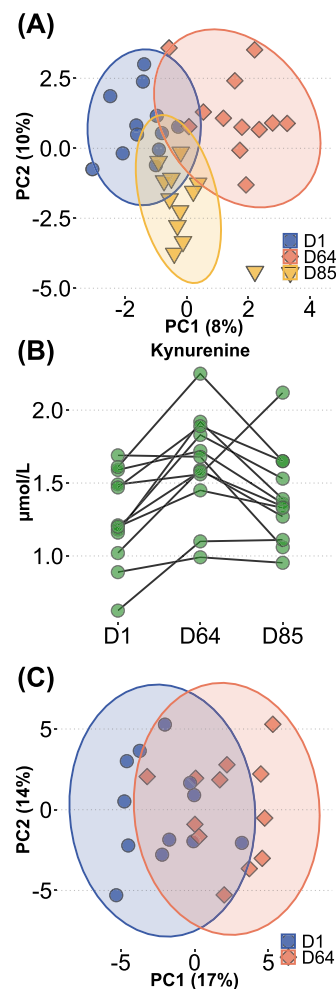


FIGURE 2 Change of metabolomic profiles over time in the treatment group. (A) Principal component analysis (PCA) plot of the SPLS-DA model built to discriminate the three time points in the treatment group. D1, D64, and D85 are in blue circles, orange diamonds, and gold triangles, respectively. Ellipses represent the 95% confidence interval for each time point. (B) Change in kynurenine levels ($\mu\text{mol/L}$) over time (D1, D64, and D85) in the treatment group. Black lines connect the different time points of a single patient. (C) PCA plot for D1 (blue circles) and D64 (orange diamonds) for the treatment group. Ellipses represent the 95% confidence interval for each time point.

Treg number was significant ($Q^2 = 0.51$, $p = 0.01$). Eight metabolites were selected to build this model: C14:2, C14:2-OH, C5-DC (C6-OH), L-DOPA, spermidine, spermine, lysoPC a C18:2, and PC aa C44:3.

As the Treg number was not modified within the placebo group, we focused on the treatment group for the correlation between Treg number and metabolites at D64. Three metabolites were correlated with the Treg number at D64 in the treatment group: C12:1 ($r = 0.80(10)$, raw p -value = 0.002), PC aa C32:0 ($r = 0.69(10)$, raw p -value = 0.013), and PC aa C34:3 ($r = 0.59(10)$, raw p -value = 0.044). We also found a significant PLS model ($Q^2 = 0.42$, $p = 0.001$) that included: alanine, arginine, glutamine, lysoPC a C20:4, PC aa C32:0, PC aa C32:1, and PC aa C34:3.

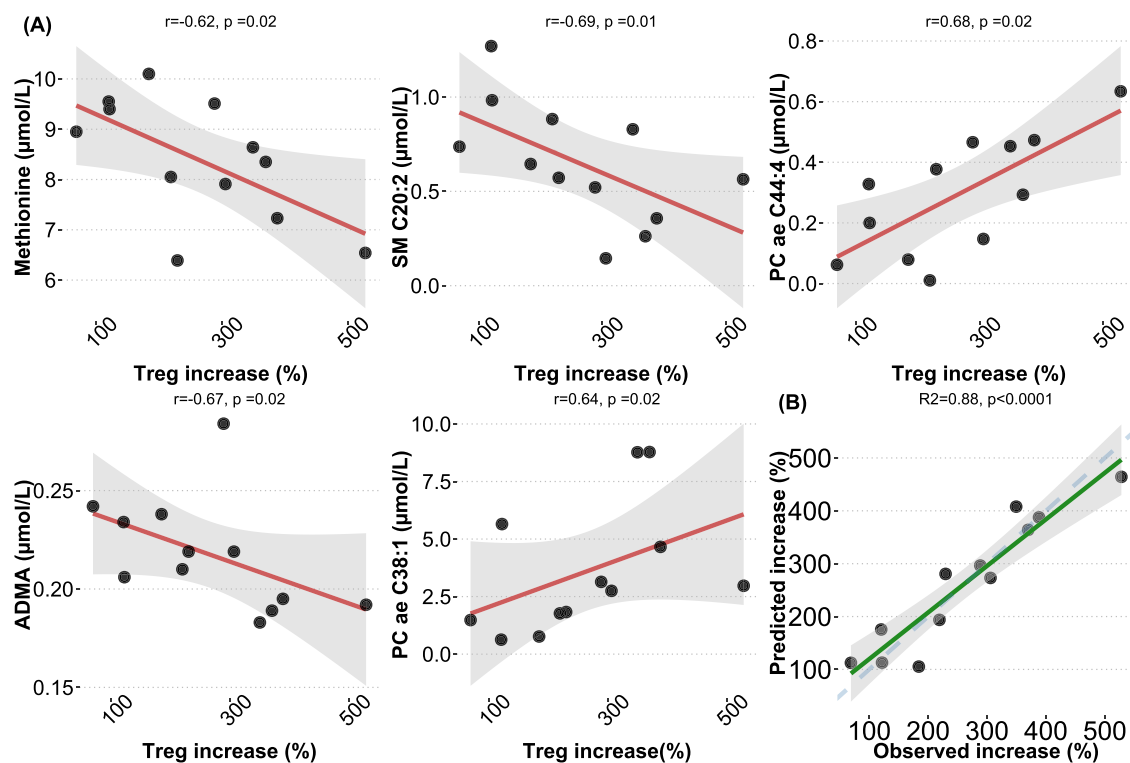


FIGURE 3 Association between baseline metabolome and the percent increase of Treg count in the treatment group. (A) Correlations between the percentage increase of Treg number and metabolite levels at D1. Raw p -value < 0.05 . Red lines show the line of best fit. Gray bands represent the 95% confidence level intervals. (B) Correlation between the observed percent increase in Treg number and the predicted percent increase determined using PLS model. The green line shows the line of best fit. Gray band represents the 95% confidence level interval. The blue dashed line represents a perfect correlation ($r = 1$). Abbreviation: ADMA, asymmetric dimethylarginine.

Prediction of Treg increase after Id-IL-2 administration from baseline metabolome

As we found a correlation between Treg number and metabolites at each time point, we evaluated the ability of the metabolome at D1 to predict the percent increase in Treg number from D1 to D64. Five metabolites were correlated with the Treg increase before FDR correction: SM C20:2, PC ae C44:4, asymmetric dimethylarginine (ADMA), PC ae C38:1, and methionine (Figure 3A). The permutation test was significant, and the CV showed an R^2 and Q^2 of 0.52 and 0.51, respectively, within the first component. Forty-four metabolites were selected to build the model after keeping only features with a VIP value > 0.8 within the first component (see details and VIP values in Table S5). We then used this model to predict the percent increase of Treg number from D1 to D64. The predicted values were highly correlated with the observed values ($R^2 = 0.88$ (10), $p < 0.0001$) (Figure 3B).

DISCUSSION

Ld-IL-2 has emerged as a therapeutic candidate, and its safety and tolerance have been reported in ALS patients in our phase 2a IMODALS

trial.¹⁷ To explore the variability of drug response in this cohort and complement the study by Giovannelli et al.¹⁸ we proposed a pharmacometabolomics approach to provide new insight into metabolic changes occurring in patients treated with Id-IL-2 and their relationship with the Treg response.

Benefit of pharmacometabolomics

Understanding pharmacometabolomics²⁰ can be particularly important in ALS due to the failure of numerous clinical trials for drugs to treat this disease, and the high heterogeneity of treatment responses could mask potential efficacy in some patients. One of the strengths of the IMODALS cohort is the opportunity it provides to explore different biomarkers from patient material (e.g., biological fluids, RNA, etc.) that may complement standard biological and clinical findings. We characterized the metabolome at different time points in both the treatment and placebo groups at baseline (D1), the peak of treatment (D64), and the end of treatment (D85). Thus, the design of this trial allowed for the identification of specific metabolic changes caused by Id-IL-2 treatment, as suggested by the ability of our models to discriminate the different time points in the treatment group.

Metabolic alterations associated with Id-IL-2 administration

Among the Id-IL-2-induced metabolite modifications, kynurenine displayed an interesting pattern. Kynurenine is formed by the catabolism of tryptophan by indoleamine 2,3 dioxygenase 1 (IDO-1).²³ The kynurenine pathway (KP) is activated during inflammation to provide NAD⁺ to immune cells, and some of its intermediates have immunomodulatory (pro- and anti-inflammatory depending on the intermediates) effects.^{24,25} KP represents a link between inflammation and metabolic alterations²⁶ and is one of the promising pathways recently investigated in neurodegenerative disease, including ALS.²⁷⁻³⁰ Our results are consistent with the literature as IL-2 has long been reported to induce IDO-1 activity,³¹ which could explain the elevation of kynurenine found in the treatment group following administration of Id-IL-2. Moreover, in the same cohort, Giovannelli et al. reported an upregulation of *IDO1* transcription between D1 and D64 in the treatment group, reinforcing the reliability of our results and underlining the value of a combined metabolomic and transcriptional approach to explore the drug's mechanism of action.¹⁸ In regard to the emerging role of this pathway, we can take advantage of the upregulation of kynurenine levels induced by Id-IL-2. Indeed, we could attempt to modulate its metabolism toward the formation of neuroprotective intermediates, such as kynurenic acid, with kynurenine monoxygenase inhibitors.³² These findings could open new therapeutic opportunities for ALS.

Among the alterations of other metabolites induced by Id-IL-2 treatment, we observed a decreasing trend for various PCs (Table S4). This could be explained by the reported role of IL-2 in the activation of protein kinase C (PKC).^{33,34} This activation would appear to be mediated by the hydrolysis of PCs by phospholipase C.³⁴

Promising prediction of Treg increase from baseline metabolome

Although an elevation of Treg number was observed for all patients in the treatment group,¹⁷ the scale of this elevation was heterogeneous. It is, therefore, important to identify factors influencing this variability, as done with transcription expression.¹⁸ Baseline metabolome was able to predict the increased number of Tregs at D64 in the treatment group. Among the important metabolites for our model, methionine uptake by the SLC43A2 transporter has recently been reported to be crucial for Treg viability upon IL-2 deprivation.³⁵ We found an inverse correlation between methionine levels at baseline and the increase in Treg number. We can hypothesize that with less circulating methionine, Tregs would be more prone to respond to Id-IL-2 treatment.

ADMA, generated from the methylation of arginine residues, is an endogenous inhibitor of nitric oxide production.³⁶ Due to its function, this molecule has been largely investigated in cardiovascular diseases and has emerged as a risk factor of cardiovascular events via endothelial dysfunction.³⁷ Moreover, this molecule would also appear to play

a role in inflammation.³⁸ ADMA can upregulate IL-6 production, as reported in an in vitro study,³⁹ but the opposite has also been found in a rat model.⁴⁰ As IL-6 is known to inhibit Treg differentiation,⁴¹ it could explain the inverse correlation we found here between ADMA levels at D1 and the percent increase in Treg number. Of note, ADMA was found to be correlated with IL-2 levels in a cohort of patients suffering from periodontal disease.⁴² In our cohort, we found a modest increase following the administration of Id-IL-2 (nonsignificant fold change = 1.14 between D1 and D64).

Sphingomyelins are structural phospholipids that can be hydrolyzed into ceramides through sphingomyelinase activity.⁴³ We suggest that the inverse correlation between baseline SM C20:2 and the increase in Tregs could be explained by an increase in ceramide production as ceramide can negatively regulate Tregs via intracellular signaling.^{44,45} As for the positive correlation between Treg number and PC, we could suggest that PKC activation following hydrolysis of PCs could contribute to Treg induction.⁴⁶ Determining the intracellular levels of these metabolites would be of interest to explore their correlation with the Id-IL-2 response.

Limitations and future directions

Our pharmacometabolomic approach was limited to the biological response to Id-IL-2, so we will consider the clinical response as an endpoint in future studies as there was a lack of significant deterioration of clinical features over the phase 2a trials in all groups.¹⁷ Another limitation of our study is the use of a targeted metabolomic kit focused on amino acids and lipids, which, therefore, does not cover all metabolic pathways. For example, based on our results, exploration of ceramides and KP intermediates would have been relevant. This study was performed under the optimum conditions for a metabolomics approach with an extensively validated, quantitative metabolomics kit. This method provides confidence and gives us an overview of the key mechanisms that we must continue to examine in further studies and leads us to utilize an untargeted approach.

The major limitation of our study is the limited number of patients, which is due to the nature of the phase 2a IMODALS study. To reduce this limitation, we used component-based approaches such as sPLS-DA and PLS that are suitable when the number of explanatory variables is much greater than the number of observations. These approaches reduce the dimensionality of a multivariate dataset to a few principal components with minimal loss of information. They are also suitable when there is a lot of collinearity between variables. However, the selection process used cannot guarantee the absence of confounding variables. With the lack of external validation and statistical power, our models are prone to overfitting. Thus, our results can highlight potential metabolites involved in the Id-IL-2 response, but mainly serve as a proof of concept for a subsequent study involving more patients. The IMODALS study was followed by the phase 2b MIROCALS study (NCT03039673), which included a greater number of patients with a longer follow-up. It would be interesting to conduct a pharmacometabolomics approach (covering a higher number of metabolites,

including ceramides and KP intermediates) on this cohort to (i) replicate and increase the robustness of this study and (ii) explore metabolic changes linked to the clinical response to Id-IL-2. The replication of our results and the determination of an optimal threshold target for the rising number of Tregs could provide an opportunity to evaluate the benefits of a pharmacometabolomics-guided Id-IL-2 dose in the context of ALS. Our study also prompts for further studies evaluating the intracellular metabolome of Tregs and its interaction with the transcriptome and proteome of these cells following the response to Id-IL-2 in ALS.

CONCLUSION

Our pilot study provided proof of concept of a pharmacometabolomics approach to elucidate the metabolic effects Id-IL-2 in the ALS environment. We identified alterations associated with Id-IL-2 administration, particularly in PCs and the KP. The changes in the KP are supported by the upregulation of genes involved in this pathway that have previously been reported in the same cohort. These findings underscore the intricate interplay between immune modulation and metabolic pathways in ALS pathogenesis and treatment response. We also identified potential biomarkers associated with treatment response, such as methionine, ADMA, SM C20:2, and PCs. These data could result in advances toward personalized medicine in ALS based on patient-specific metabolomic profiles, but also contribute to the understanding of ALS heterogeneity. Pharmacometabolomics could be a complementary tool to genomic and transcriptional data for drug characterization and lead to a systems pharmacology approach. We believe multi-omics profiling is ready for consideration as a secondary outcome in clinical trials in ALS and other neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

W.C. and R.J.-M. were involved in clinical data collection. G.B. and H.B. were involved in conceptualization. G.B., H.B., and P.E. were involved in designing the specific laboratory tests of the study. C.B., P.E., and H.B. were involved in specific laboratory data collection. C.B., H.B., and H.A. were involved in data management and data analysis. H.B., G.B., and H.A. were involved in data interpretation. H.B. and H.A. were involved in writing the original draft. W.C., J.-L.V., C.G., M.L., R.J.-M., S.S., C.S., C.M., J.K., P.S., A.M., J.D.V., A.A.-C., T.T., P.N.L., and G.B. were involved in the initial IMODALS project. All authors critically reviewed the manuscript.

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COMPETING INTERESTS

H.A., C.B., P.E., C.R., J.-L.V., R.J.-M., S.S., C.S., C.M., J.D.V., P.V., W.C., and H.B. have nothing to disclose. A.A.-C. reports consultancies or

is on the advisory boards for Amylyx, Apellis, Biogen, Brainstorm, Clene Therapeutics, Cytokinetics, GenieUs, GSK, Lilly, Mitsubishi Tanabe Pharma, Novartis, OrionPharma, Qualis, Sano, Sanofi, and Wave Pharmaceuticals. J.K. reports grants from The Nimes University Hospital Center (CHU Nimes) and grants from EU HORIZON 2020, during the conduct of the study. J.K. also reports grants from MND Association and My Name's 5 Doddie Foundation outside the submitted work. P.C. serves on scientific and advisory boards for Amylyx, Biogen, VectorY, Cytokinetics, Zambon, Ferrer, and Mitsubishi Tanabe Pharma. G.B. reports grants from the French Health Ministry (PHRC-I), ARSLA, and EU HORIZON 2020, during the conduct of the study. G.B. has a patent (WO 2012123381 A1) with royalties paid to Assistance Publique Hopitaux de Paris (APHP), Institut National de la Sante et de la Recherche Medicale INSERM, and Sorbonne Universite. G.B., T.T., P.N.L., M.L., C.G., P.S., J.K., and A.M. have a patent (B75649EPD40021) pending. A.M. reports grants from EU HORIZON 2020, MND Association UK, NIHR, MRC, NIH, Barts and the London Charity, and UCB Pharma. A.M. has acted as a consultant for Roche, Pfizer, and Accure Therapeutics. He has worked as a site investigator and Work-Package Lead on clinical trials funded by EU2020. He reports Biomarkers data licensing to Biogen and Clinical Trial data to ILTOO. P.S. reports grants from EU HORIZON 2020, Sheffield component, and MIROCALS (633413), outside of the submitted work.

DATA AVAILABILITY STATEMENT

Data are available from the authors with the permission of the sponsor (DRC@chu-nimes.fr)

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PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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