Metabolic profile evolution in relapsed/refractory B-cell non-Hodgkin lymphoma patients treated with CD19 chimeric antigen receptor T-cell therapy and implications in clinical outcome

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Abstract

Plasma metabolomics analysis was performed on 44 patients with relapsed/refractory B-cell non-Hodgkin lymphoma (r/r/B-NHL) infused with approved CD19 chimeric antigen receptor (CAR) T-cell products at the time of pre-lymphodepletion (PLD) and at day +1 (D1), D7, and D30 after CAR T-cell infusion. At the PLD time point, a metabolic profile characterized by high lipoproteins and lactate and low glucose contributed to poor outcome prediction in association with high lactate dehydrogenase levels. At D1, higher plasma levels of lipid metabolism products and lower glucose and glycoproteins levels were observed in tisa-cel-compared to axi-cel-treated patients. At D30, discriminant analysis found two clusters in a subgroup of patients, one with complete response lasting 1 year after therapy, and another who relapsed within 1 year (relapsed >D30). This latter showed a higher content of N-GlycA, a known biomarker of systemic inflammation that is also correlated with C-reactive protein in our case setting of relapsing patients. Our data show complex metabolomic changes that track the evolution of the disease and drug activity in the first 30 days of CAR T-cell therapy. Conceivably, a pro-inflammatory drift may be linked to a forthcoming disease relapse in CAR T patients.

Introduction

Chimeric antigen receptor (CAR) T-cell therapy is an innovative approach for patients affected by relapsed or refractory aggressive B-cell non-Hodgkin lymphoma (r/r B-NHL) who either failed to respond to autologous stem cell transplantation or are ineligible for it.[1] Some pre-treatment factors have been identified to be associated with relapse or progression, such as elevated lactate dehydrogenase (LDH),[2] tumor burden measured either by the lesion diameter or by the total metabolic tumor volume on positron emission tomography and computed tomography (PET-CT) scan, and more than one extranodal site involved.[3] Our study aims to investigate the potential clinical utility of a nuclear magnetic resonance (NMR)-based metabolomic approach to differentiate patients who achieve a durable response to CAR T-cell therapy from those who do not respond or progress after an early metabolic response at 1 month after CAR T-cell therapy. In this real-life study, plasma samples of 44 r/r B-NHL patients treated with Italian Medicines Agency (AIFA)-approved CD19 CAR T-cell products were analyzed with a metabolomic approach based on NMR spectroscopy at pre-lymphodepletion (PLD) and after infusion.

Methods

Patients

This is a prospective observational tissue study performed in patients affected by r/r B-NHL after CD19 CAR T-cell therapy with axi-cel and tisa-cel. Only patients with diffuse large B-cell lymphoma (DLBCL), DLBCL transformed from follicular lymphoma (tFL), high-grade B-cell lymphoma (HGBCL), and primary mediastinal B-cell lymphoma (PMBCL) were included. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were graded according to the American Society for Transplantation and Cellular Therapy (ASCT) criteria.[4] All eligible patients were treated at IRCCS AOU Bologna, after signing a written informed consent. The study was approved by the local Ethical Committee in agreement with the Declaration of Helsinki and registered at *clinicaltrials gov. Identifier: NCT04892433*.

Sample collection and assessment of biochemical parameters

Blood samples were collected at the PLD time point and day +1 (D1), D7, and D30 after CAR T-cell infusion, when available. Plasma separation was performed within 4 hours after blood sample collection by centrifugation at 1,000xg for 15 minutes at room temperature. Plasma aliquots were collected and stored at -80°C. All the serum biochemistry reported in the study was assessed according to the standard practice at the IRCCS AOU Bologna analysis service.

Nuclear magnetic resonance measurements and data processing

The present research follows the requirements and recommendations for the handling and processing of blood plasma for metabolomics analysis in the pre-examination processes according to ISO 23118:2021. We performed a study assessing the compliance of the datasets in the Metabolomics Workbench repository.[5] Plasma sample preparation for NMR measurements is reported in the *Online Supplementary Appendix*.

Statistical analysis

Unsupervised principal component analysis (PCA), and supervised orthogonal partial least squares discriminant analysis (OPLS-DA) were performed on NMR data using Simca 14.0 software (SIMCA® https://www.sartorius.com/en). For all the statistical models performed on NMR data, the goodness-of-fit (R^2X), the proportion of the variance that is explained by the model (R^2Y), and the goodness-of-prediction of the model (Q^2) parameters were evaluated, providing reliability and an internal measure of consistency between the original and (7-fold) cross-validated predicted data.[6] Moreover, for OPLS-DA models, variable importance for projection (VIP) and loadings vectors scaled as correlation poso(corr) are also considered for discriminating variables.[7]

Both univariate and multivariate statistical analyses were performed using MetaboAnalyst software[8] and the SPSS/PC-computer program (SPSS, Chicago, IL, USA).
Summary statistics of all explanatory variables are reported for the whole population as required. Association between PC1 and PC2 obtained at the PLD time point and patients' features included in Table 1 was represented using boxplots

Table 1. Patient and disease characteristics before CAR T-cell therapy.

Characteristics	Total N=44
Median age in years (range)	60.5 (21-70)
Sex, N (%)	
Male	33 (75)
Female	11 (25)
BMI, N (%)	
<25	15 (34)
≥25	29 (66)
ECOG, N (%)	
0	35 (79.5)
≥1	9 (20.5)
Diagnosis, N (%)	
DLBCL	21 (47.7)
HGBCL	2 (4.6)
PMBCL	6 (13.6)
tFL	15 (34.1)
Bulky disease, N (%)	
NA	1 (2.3)
Yes	28 (63.6)
No	15 (34.1)
Staging, N (%)	
I-II	12 (27.3)
III-IV	32 (76.7)
IPI score, N (%)	
NA	6 (13.6)
0-2	23 (52.3)
≥3	15 (34.1)
Bridging therapy, N (%)	
Chemotherapy-based	16 (36)
Cellular infused product, N (%)	
Axi-cel	24 (55)
Tisa-cel	20 (45)
CAR-hematotox score, N (%)	
0-1	35 (79.5%)
≥2	9 (20.5%)
CRS, N (%)	
Yes	38 (86.4)
No	6 (13.6)
ICANS, N (%)	
Yes	14 (31.8)
No	30 (68.2)

CAR: chimeric antigen receptor; ECOG: Eastern Cooperative Oncology Group performance scale; BMI: body mass index; DLBCL: diffuse large B-cell lymphoma; tFL: transformed follicular lymphoma; HGBCL: high-grade B-cell lymphoma; PMBCL: primary mediastinal B-cell lymphoma; IPI: International Prognostic Index; NA: not available; CRS: cytokine release syndrome; ICANS: immune cell-associated neurotoxicity syndrome.

and analyzed by Kruskal-Wallis tests, with Bonferroni correction for multiple comparisons. Statistical significance was set at $P \leq 0.05$. Given the presence of outliers and the non-normality of the data, correlation is measured by Spearman's coefficient. All variables significantly associated with clinical outcomes were evaluated in a multiple Cox regression. In particular, stepwise methods with 0.1 significance level including all covariates were performed to select the best subset of predictors. Finally, predictive performance of the model was measured according to the resulting operating characteristic (ROC) area under the curve (AUC).

Results

Patient characteristics

All patients (N=44; Table 1) had r/r B-NHL: 36 (81.9%) DLBCL (including 15 tFL), six (13.6%) PMBCL and two patients (4.5%) with HGBCL. Twenty-four patients (55%) received axi-cel and 20 (45%) received tisa-cel after a median of three prior lines of treatment. The average age was 60 years (range, 21-70). All patients underwent CAR T-cell infusion following standard lymphodepletion chemotherapy with cyclophosphamide (250-500 mg/m^2) and fludarabine (25-30 mg/m^2) administered intravenously on days -5, -4, and -3 according to standard practice. Among 44 patients who underwent infusion, 38 (86.4%) developed CRS (any grade). Four patients (9.1%) experienced grade ≥3 CRS. Within 1 month of CD19 CAR T-cell infusion, 14 (32%) of 44 patients developed ICANS of any grade. Six patients (13.6%) developed ICANS grade ≥3. Two patients (4.5%) died of fatal neurotoxicity with diffuse cerebral edema (ICANS, grade 5). At 1 month post-infusion, the overall response rate (ORR) was 64%: 14 (32%) patients achieved complete remission (CR), and 14 (32%) patients achieved partial remission (PR); eight (18%) patients achieved progressive disease (PD) and six (14%) had stable disease (SD). At the latest available follow-up (1 year), 13 patients were in CR and three in PR. The others (N=26) had experienced progression during the course of the follow-up.

Median follow-up was 364 days for overall survival (OS) and 147 days for time-to-progression (TTP) and progression-free survival (PFS).

Metabolic profile of relapsed/refractory B-cell non-Hodgkin lymphoma patients before and after receiving CAR T-cell therapy

We performed a PLD metabolomic profile of 44 patients of whom 22 also at D1, D7 and D30 time points after CAR T-cell infusion (see the *Online Supplementary Appendix*; *Online Supplementary Figure S1A*).

A representative ^{1}H NMR spectrum and the chemical shifts (δ) and assignments of metabolites resonances of the acquired ^{1}H NMR spectra are provided in the *Online Supplementary Appendix*; *Online Supplementary Figure S1B*; *Online Supplementary Table S1*).

Firstly, we focused on pre-treatment metabolic profile and the PCA model was built using the first two principal components (PC) that explained 34.6% and 11.7% of the total variance in an eight-components model (*Online Supplementary Figure S2A*). NMR signals related to lipoproteins and glucose were found at positive and negative values of the first PC1 component, respectively (*Online Supplementary Figure S2B*). Moreover, the contribution of loadings related to lactate and glycoproteins, specifically N-GlycA which refers to a subset of glycan N-acetylglucosamine residues (NMR signal at 2.05 ppm, related to -COCH3 acetyl groups of sera glycoproteins), were both found at positive values of PC2 (*Online Supplementary Figure S2B*).

We performed the Kruskal-Wallis multiple comparisons between the first two PC and anthropometric and clinical parameters included in Table 1.

As reported in Figure 1, the PLD metabolic profile described by PC1 was different according to diagnosis (P=0.039). We also explored the potential correlation with variables associated with inflammatory status and/or tumor burden, namely triglycerides, LDH, fibrinogen, ferritin, and C-reactive protein (CRP) measured at PLD (Figure 2A). PC1 was positively correlated with triglycerides (r=0.81; P<0.001) (Figure 2A).

Cox regression was then used in TTP to determine the influence of multiple variables on the outcome. Starting with the whole set of covariates, stepwise selection methods on the whole set of patients identified PC1 (hazard ratio [HR]=1.052, 95% confidence interval [CI]: 1.012-1.094; P=0.01) and LDH (HR=1.001, 95% CI: 1.000-1.003; P=0.03) as predictors. The resulting Cox model confirmed the predictive power of PC1 since its addition into the model gave better predictions in terms of ROC-AUC with respect to the model with lactate alone (AUCLDH¬=0.61 and AUC¬LDH+PC1¬=0.81, respectively; Figure 2B).

Based on the contribution of the loadings for the first two PC (see *Online Supplementary Figure S2B*), the box-whisker plots report the distribution of discriminant metabolites according to the last available follow-up (CR, N=13 *vs*. PD, N=26 *vs*. PR, N=3; *Online Supplementary Figure S2C*). Specifically, we observed higher levels of lipoproteins and lactate and lower amounts of N-GlycA, succinate and glucose in PD and PR patients than in CR ones (*Online Supplementary Figure S2C*).

A supervised analysis (OPLS-DA) showed a good separation between the most representative patient's groups (CR, N=13 *vs*. PD, N=26; Figure 2C). The most evident differences in metabolites between the patient groups were observed in the levels of lipoproteins/lipids and lactate, which were higher in PD than CR, as well as in the level of glucose, which was lower in PD than CR (Figure 2D; *Online Supplementary Table S2*).

Next, we focused on a sub-cohort of 22 patients whose plasma samples were available to test differences in the metabolomic profiles across time after CAR T-cell infusion (D1, D7, and D30) (Figure 3A, C, D) according to the infused

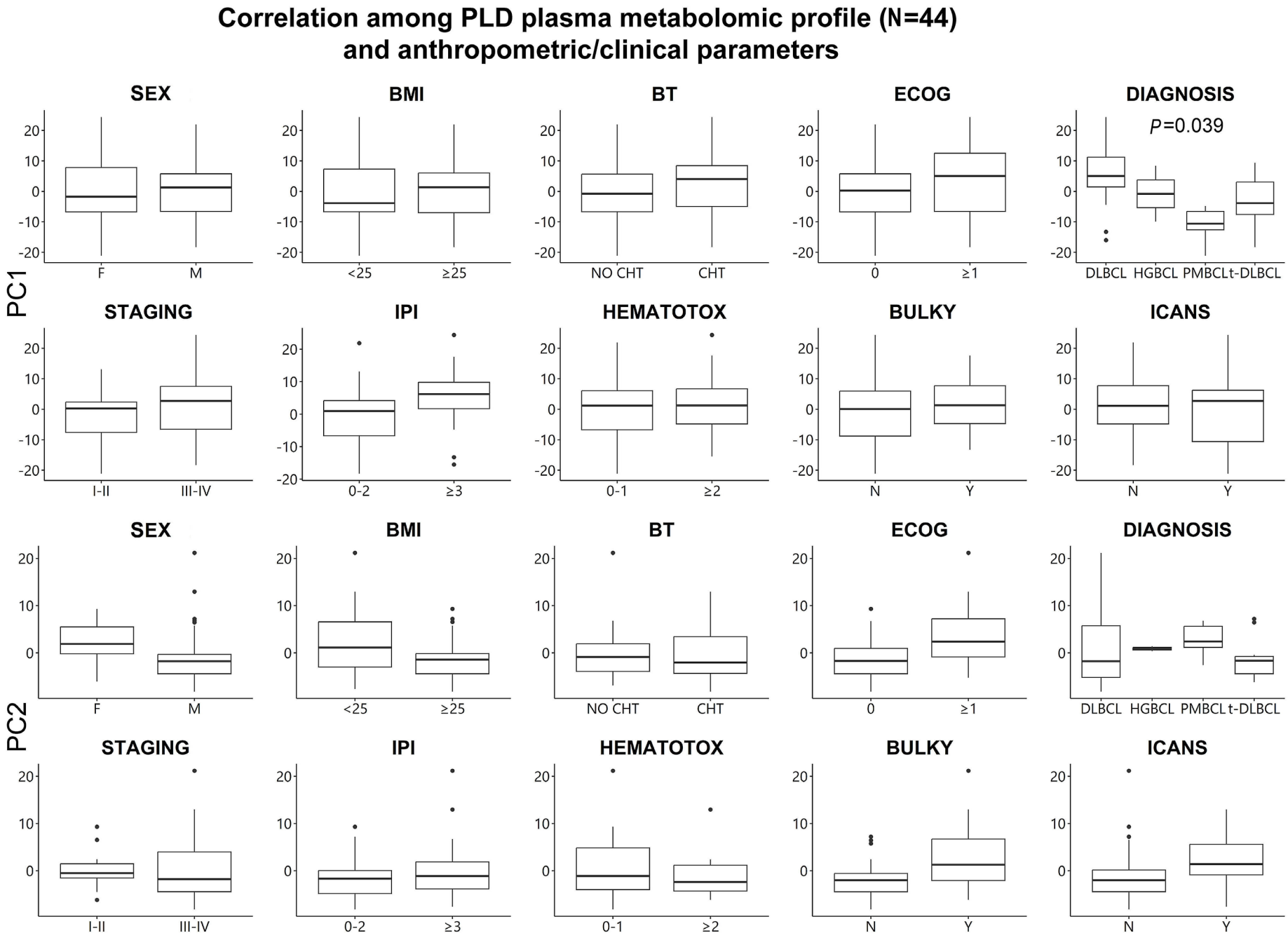

Figure 1. Pre-lymphodepletion plasma metabolic differences correlated with anthropometric and clinical parameters collected at pre-lymphodepletion time point. Relationships among principal components 1 and 2 (PC1 and PC2) and sex, body mass index (BMI), bridging therapy (BT), Eastern Cooperative Oncology Group performance scale (ECOG), diagnosis, staging, International Prognostic Index (IPI), HEMATOTOX, bulky, immune cell-associated neurotoxicity syndrome (ICANS). *P* values ($P<0.05$) are calculated by the Kruskal-Wallis test adjusted with Bonferroni correction for multiple comparisons; PLD: pre-lymphodepletion.

product (axi-cel *vs*. tisa-cel). No differences in the metabolic profile at the PLD time point were observed (*Online Supplementary Figure S3*), but specific metabolic signatures were identified among patients, specifically at D1 (Figure 3A). These differences progressively disappeared over time (Figure 3C, D). As reported in Figure 3B and *Online Supplementary Table S3*, at D1, the S-line plot showed higher levels of lipoproteins, 3-hydroxybutyrate (3-OH butyrate), and acetate, and lower levels of glucose and GlycA, which refers to a subset of glycan N-acetylglucosamine residues in tisa-cel-treated patients compared to those infused with axi-cel.

Moreover, time-dependent metabolic changes were observed from D1 to D30 in patients stratified according to the last available follow-up (Figure 4). Specifically, a reduced N-GlyA and lipoproteins/lipids content was observed in patients who achieved CR (Figure 4A, B) compared with PD patients who showed an increased content of succinate/pyruvate and lactate (Figure 4C, D). This observation suggests that plasma can efficiently mirror the metabolic pattern of lymphoma cells that survive within 30 days after CAR T-cell therapy.

Considering this finding, we considered a subgroup of patients in whom PET/CT scans showed a CR/PR (N=14) at D30 time point. Discriminant analysis found two clusters of patients, one with CR lasting 1 year after therapy (stable CR day >30), and another who relapsed within 1 year (relapsed day >30) (Figure 5A). Metabolic differences in the content of N-GlycA, histidine, glutamate, phenylalanine and branched-chain amino acids (BCAA) (leucine, isoleucine and valine) were observed between relapsed day >30 and stable CR day >30 (Figure 5B, C; *Online Supplementary Table S4*). The

unpaired *t* test analysis showed that N-GlycA and histidine were significantly enriched in relapsed day >30 patients (Figure 5C and D; *Online Supplementary Table S4*). Moreover, a correlation between N-GlycA and CRP was found in the case set of relapsing patients (spearman ρ=0.93**).

To further prove that glycans are associated with poor prognosis in the post-CAR T-cell infusion setting, we took advantage of a DSA-FACE-based method (*Online Supplementary Appendix*) for glycans assessment.[9] The assay allowed us to detect a subset of eight N-glycans on D30 plasma samples from an independent cohort of 20 patients who were in CR/PR on the basis of the D30 PET/CT scans. This approach gave us the possibility to calculate the GlycoAge[10] score which, in our case series, was positively correlated with CRP (r=0.58; *P*=0.01) and that showed a trend towards higher levels (*P*=0.076) in patients who relapsed at 3 or 6

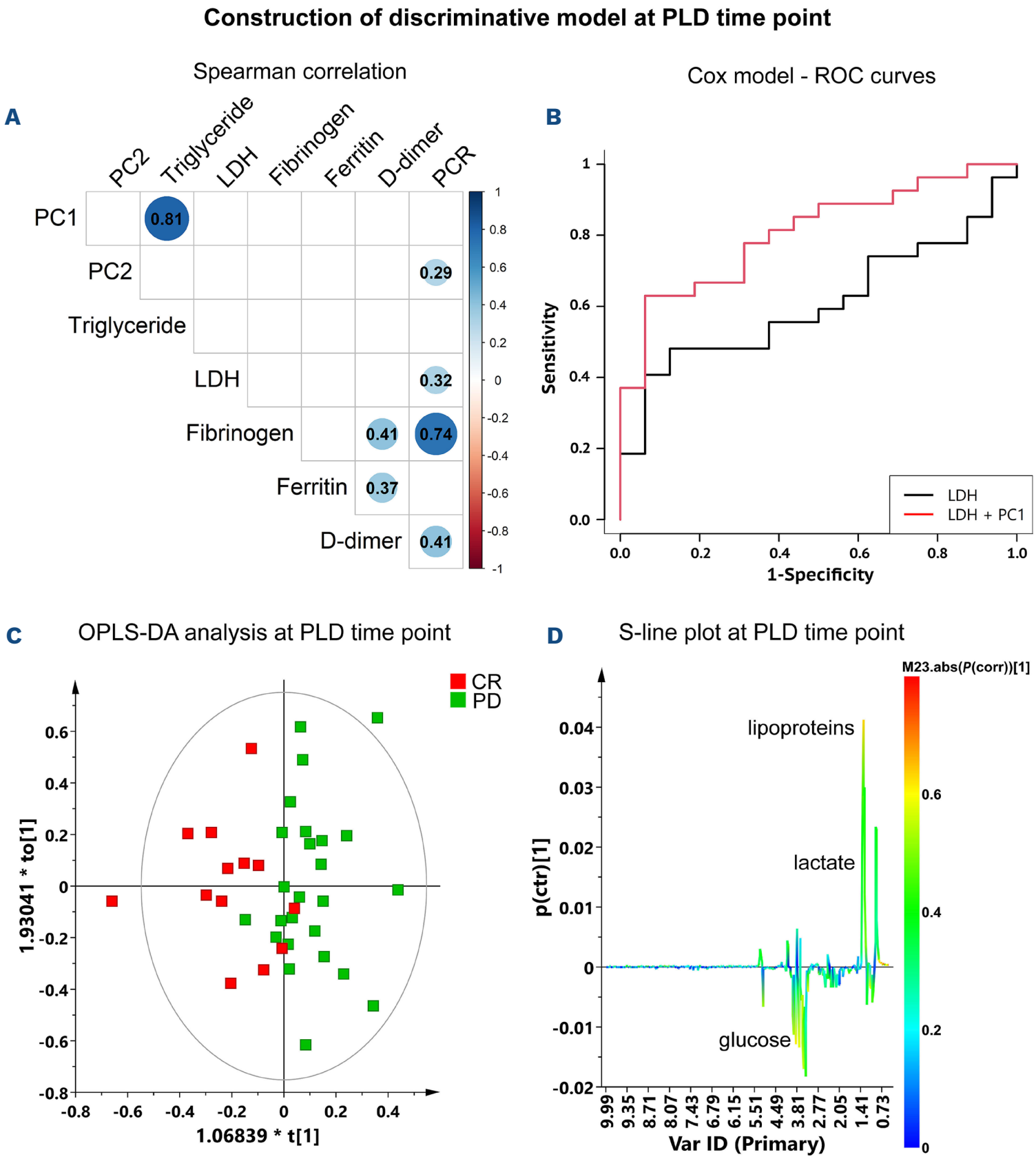

Figure 2. Pre-infusion construction of a discriminative model at pre-lymphodepletion time point. (A) Spearman r coefficient heat maps of principal components 1 and 2 (PC1 and PC2) correlated variables namely triglycerides, lactate dehydrogenase (LDH), fibrinogen, ferritin, and C-reactive protein (CRP) measured at pre-lymphodepletion (PLD) time point. (B) Resulting operating characersis (ROC) curves of the 2 models based on the whole set of patients. (C) Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot and (D) the corresponding S-line plot of PLD metabolic profile of patients stratified according to the last follow-up available after chimeric antigen receptor (CAR) T-cell infusion (CR, N=13 *vs*. PD, N=26).

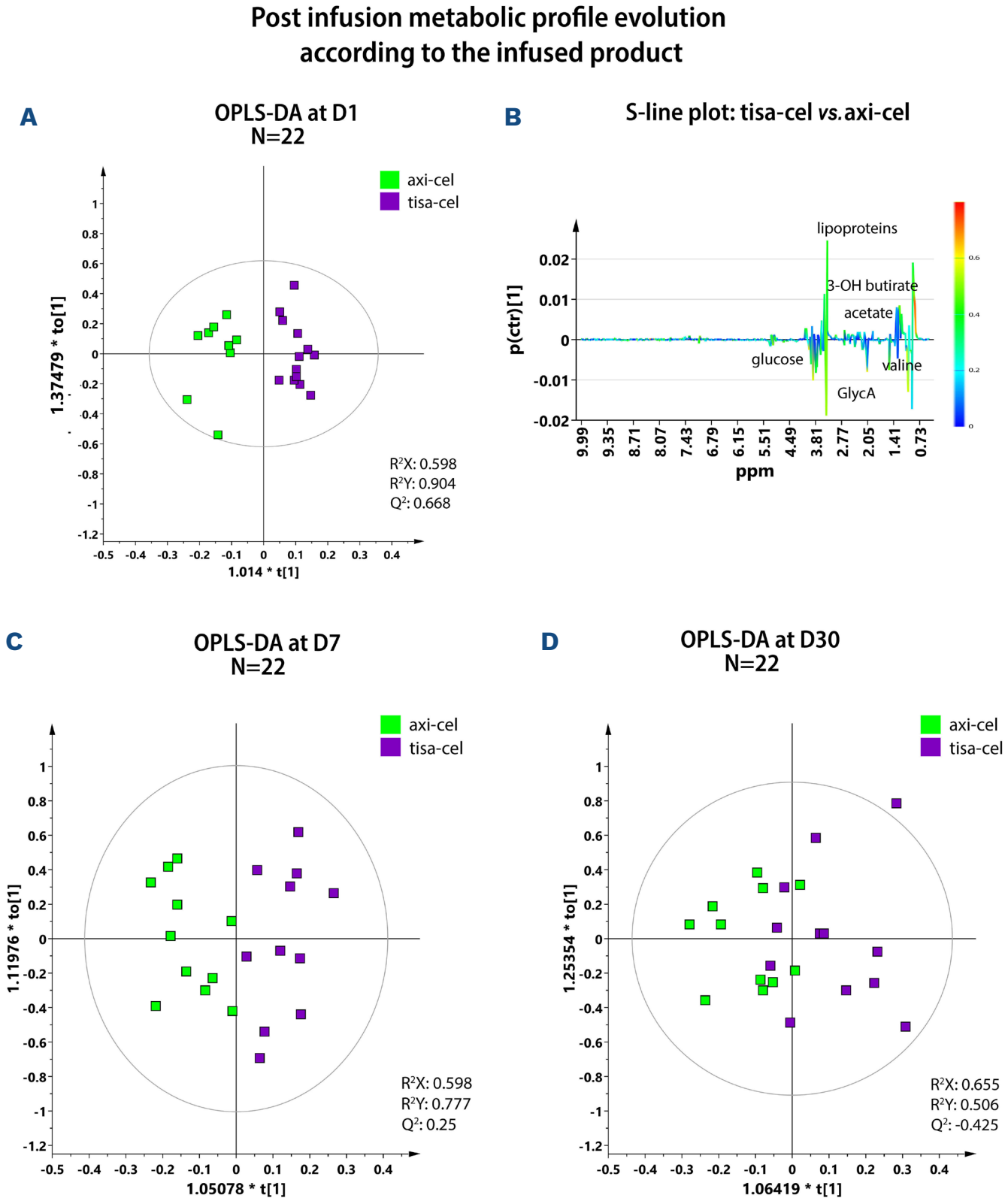

Figure 3. Post-infusion metabolic profile evolution according to the infused product. (A, C, D) Orthogonal partial least squares discriminant analysis (OPLS-DA) for day +1 (D1), D7, and D30 time points; statistical parameters of supervised OPLS-DA models for each of the considered time points. R^2X and R^2Y indicate the fraction of variance of the X and Y matrix, respectively. Q^2 is a goodness of prediction parameter representing the portion of the variance in the data predictable by the model. (B) S-line plot related to tisa-cel-treated patients *versus* those infused with axi-cel at D1 time point.

months compared to those who remained in CR up to 6 months (*Online Supplementary Figure S4*).

Discussion

Due to the high morbidity and financial costs associated with CAR T-cell therapy, it is important to identify robust biomarkers predictive of either responsiveness or resistance to treatment.

In previous studies, analysis of several biomarkers identified a significant decrease in OS in patients with elevated LDH levels in the circulation, measured both before lymphodepletion and at the time of cell infusion.[3,11,12] Results from the US lymphoma CAR T consortium evaluating axi-cel outcomes reported higher plasma levels of LDH before conditioning to be a significant predictor of lower OS.[3] Moreover, multivariate analysis of tisa-cel clinical trial reported that patients with elevated pre-CAR T LDH levels had poorer performance-free survival and OS.[11]

In this real-life study, a metabolomic approach based on NMR spectroscopy was performed on plasma samples obtained from r/r B-NHL patients treated with CD19 CAR T-cell therapy at the PLD time point and D1, D7 and D30 after infusion.

Interestingly, at the PLD time point, a metabolic profile characterized by high lipoproteins and lactate and low glucose improved the capability of high LDH levels to predict a poor outcome. Indeed, the same metabolic profile has been observed in patients who progress or lose CAR T response compared to those achieving complete remission within 1 year. Bearing in mind that cancer cells derive most of their energy from aerobic glycolysis, with the production of lactate (Warburg Effect), the metabolic profile of

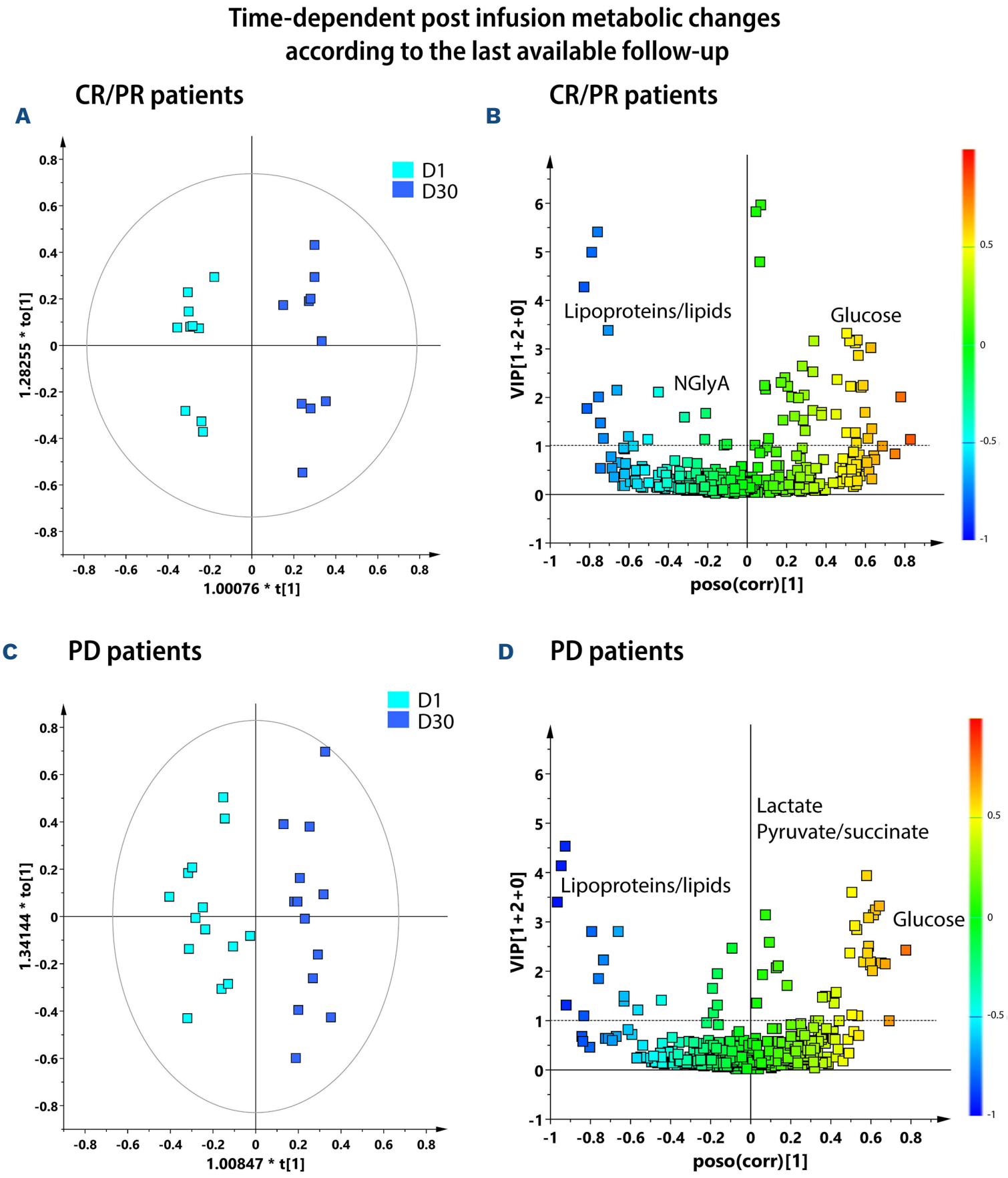

Figure 4. Post-infusion time-dependent metabolic changes according to the last available follow-up. Orthogonal partial least squares discriminant analysis (OPLS-DA) shows post-infusion time-dependent metabolic changes in (A) complete remission/partial response (CR/PR) compared to (C) progressive disease (PD) patients. (B, D) Volcano plots (poso(corr) *vs.* VIP) for the OPLS-DA models. Only the most discriminating metabolites with high VIP values are labeled. Negative poso(corr) values (left) indicate low levels, whereas positive poso(corr) values (right) indicate increased metabolite amount in patients at day +30 (D30) *versus* D1 time points.

these patients recapitulates this effect. Moreover, we can hypothesize that the high level of lipoproteins in patients who will progress or lose the response to CAR T-cell therapy could be involved in disease progression and resistance to treatment. Indeed, recent literature reports that elevated levels of cholesterol and lipids increase oxidative stress,[13] and impair T-cell immune response.[14]

In a subgroup of patients, we described the effect of the infused CAR T-cell products on the metabolic profile. Patients treated with tisa-cel showed higher plasma levels of lipid metabolism products while those treated with axi-cel showed higher levels of glucose and glycoproteins. This result could be attributable to the different co-stimulatory domains of the two CAR T-cell products. *In vitro* and *in vivo* studies have reported that 4-1BB co-stimulatory domain induces in CAR T cells a shift of metabolism towards fatty

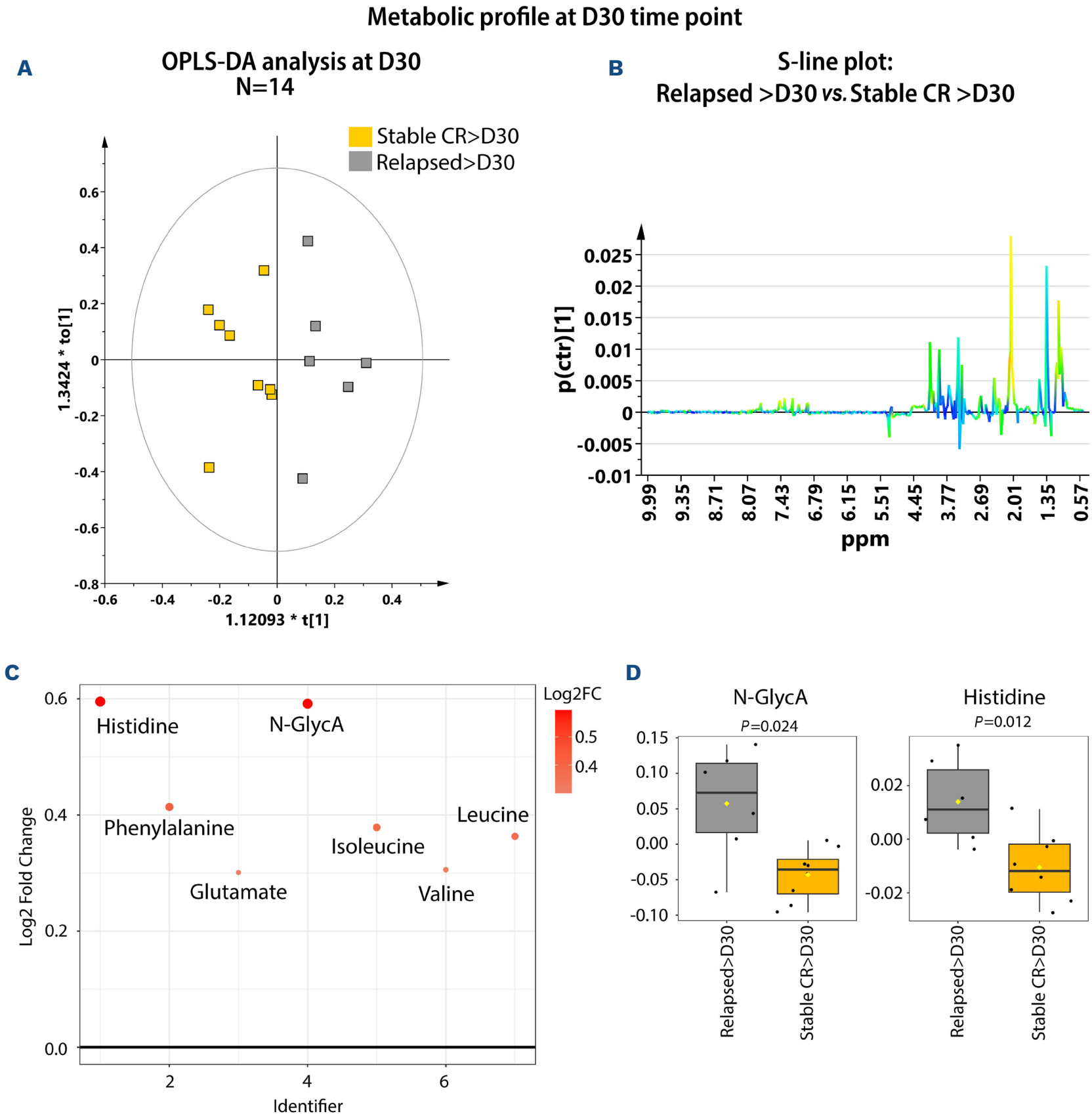

Figure 5. Metabolic profile at day +30 time point. (A) Orthogonal partial least squares discriminant analysis (OPLS-DA) for complete remission (CR) patients lasting 1 year after therapy (stable CR change [>D30]) and those who relapsed within 1 year (relapsed >D30). (B) S-line plot related to patient group, namely relapsed >D30 compared to stable CR >D30. (C) Fold change comparison of the discriminant metabolites calculated in the comparison of CR patients lasting 1 year after therapy (stable CR >D30) *versus* those who relapsed within 1 year (relapsed >D30). (D) Box-whisker plots showing the significantly discriminant metabolites between relapsed >D30 and stable CR >D30. The x-axis reports the specific metabolite group, and the y-axis the normalized peak intensity. *P* values ($P<0.05$) are calculated by unpaired *t* test.

acid β-oxidation, while CD28 domain increases glycolytic metabolism.[15,16]

According to the last available follow-up, a time-dependent change in metabolome was observed from D1 to D30. PD patients were characterized by higher lactate and pyruvate/succinate at D30 after CAR T-cell infusion. Lactate, which is produced by glucose fermentation and released from the cells into the surrounding medium, is considered an immunosuppressive metabolite as it promotes tumor progression, induces angiogenesis, stimulates amino acid metabolism, inhibits cytotoxic T, NK, and DC cells, and prevents antitumor response.[17] The higher level of lactate found in PD patients, as compared to those in CR/PR, could be related to the worsening of the patient's condition at D30 after infusion. It is known that tumor cells have a glycolytic/fermentative metabolism, consume large quantities of glucose, but produce lactate even in the presence of oxygen. In the tumor microenvironment (TME), a large amount of lactate accumulation can lead to immunosuppression via M2-like tumor-associated macrophage polarization[18,19] and myeloid-derived suppressor cells (MDSC) expansion that inhibits NK cell-mediated cytotoxic activity.[20] Moreover, an inhibitory effect of lactic acid on T-cell fitness has been suggested by Fisher K *et al*., who showed that lactic acid suppressed proliferation and cytokine production of human cytotoxic T lymphocytes, leading to a cytotoxic activity reduction.[21] Furthermore, an increase in lactic acid was found in acute myeloid leukemia (AML) patients relapsed after allogeneic-hematopoietic cell transplantation.[22] Noteworthy, the authors showed that lactic acid released by AML cells reduced glycolytic activity of T cells and led to their metabolic and proliferative dysfunction.[22]

PET-CT scan better describes the response to CAR T-cell therapy and the first evaluation is assessed at D30 after infusion. However, interpretation of the data can be complicated due to confounding factors related to cytotoxic effects induced by CAR T cells. Considering the metabolomic profile of patients with early CR/PR assessed by PET/CT at D30 time point, those who lost response at 90 or 180 days had a higher content of N-GlycA and histidine, compared to patients who remained in CR for up to 1 year. Emerging studies revealed that altered amino acid metabolism is linked to tumor growth, immunosuppression, and therapeutic resistance through the regulation of immune cell fate.[23] Noteworthy, via the action of histidine decarboxylase, which is found in cells belonging to the myeloid and lymphoid lineage, histidine forms histamine, a crucial player of inflammation.[24,25] Notably, N-GlycA is a biomarker of systemic inflammation[26,27] which correlates with circulating inflammatory proteins (e.g., IL-6, tumor necrosis factor-α, fibrinogen, CRP) that are known to be associated with adverse outcome in CAR T-cell therapy.[28] In line with this tenet, a high correlation between N-GlycA and CRP was found in the case set of relapsing patients.

According to the data showing that protein glycation increases in presence of chronic inflammation,[10,29] and that is increased in cancer bearing patients,[30] a trend towards higher GlycoAge score was found in an independent cohort of patients who relapsed at 3 or 6 months (relapsed day >30) compared to those who remained in CR for up to 6 months (CR>30).

As both NMR-detected N-GlycA and the GlycoAge[11] score are positively correlated with CRP, our data support the notion that a search for sensitive markers of cancer-related pro-inflammatory drift[30] is likely to be useful in the CAR T patients' follow-up. Though the limited sample size of our study provides only a starting point that needs to be validated in larger future studies, circulating metabolites related to systemic inflammation are likely to be important biomarkers of CAR T-cell response.

Disclosures

PLZ discloses scientific advisory board membership at Secura Bio BIO, Celltrion, Gilead, Janssen-Cilag, BMS, Servier, Sandoz, MSD, TG Therap., Takeda, Roche, EUSA Pharma, Kiowa Kirin, Novartis, ADC Therap., Incyte and Beigene; consultancy for EUSA Pharma, MSD and Novartis; speaker's bureau at Celltrion, Gilead, Janssen-Cilag, BMS, Servier, MSD, TG Therap., Takeda, Roche, EUSA Pharma, Kiowa Kirin, Novartis, Incyte and Beigene. MB has received a research grant from NEOVII. FB discloses advisory boards and speaker fees NEOVIL, Novartis, Kite, Gliead, Pfizer; Celgene; MSD, Sanofi Jazz Pharmaceuticals and BMS. The remaining authors have no conflicts of interest to disclose.

Contributions

SDM, MB, and FB designed the study. BC, FDF, ET, FB, MU, EM, MR, CP, PLZ and FB contributed patient samples and clinical data. SDM, LDC, FDC, AMG, FI, FV, MN, IS, GS, NL, DM, SNB, MT, BS, EC, MF, FPF and MB conducted the experiments and analyzed the data. SDM, LDC, AMG, MB and FB wrote the article. SDM, FDC, AMG, CP, PG and MB revised the manuscript. All authors read and agreed to the final version of the article.

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Data-sharing statement

This study is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website https://www.metabolomicsworkbench.org where it has been assigned the study ID: ST003205. The data can be accessed directly via its project DOI: http://dx.doi.org/10.21228/M8G143.

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