Associations between socioeconomic status and bispecific LV20.19 CAR T-cell therapy outcomes

Chimeric antigen receptor (CAR) T-cell therapy utilization has risen sharply in recent years, becoming a standard approach for refractory B-cell malignancies.[1] Social disparities contribute independent risk for people with cancer, an effect not fully explained by insurance status or access to care.[2] However, this relationship and its biological mechanisms have yet to be examined among CAR T-cell therapy recipients. It is hypothesized that the inflammatory stress response associated with conditions of low socioeconomic status (SES) is a mechanism underlying the impact of social health on medical outcomes.[3,4]

Importantly, proinflammatory cytokines are associated with the most common CAR T side effects, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). In fact, symptoms from CAR T-cell therapy are associated with neuroinflammatory markers, including cytokines and circulating kynurenine metabolites, that are also associated with worse patient reported outcomes (PRO) and low SES.[5-8] Here, we report the association of SES and patient response to novel bispecific anti-CD20 and anti-CD19 (LV20.19) CAR T-cell treatment on a molecular-to-clinical scale by assessing differences in annual income and inflammatory cytokine levels, kynurenine pathway metabolites, PRO, and clinical outcomes.

The current study population (n=15) is derived from the previously reported parent study (n=22) evaluating patients with relapsed, refractory B-cell malignancies treated with LV20.19 CAR T cells on a phase I/Ib clinical trial (*clinicaltrials gov. Identifier: NCT03019055*).[9] All participants provided written informed consent and all procedures were approved in advance by the MCW Institutional Review Board. Patient income was self-reported based on household annual income as either above (high SES) or below (low SES) the 2021 Wisconsin median of $54,660. Further details are described elsewhere.[8,9]

PRO and blood for kynurenine metabolites and cytokines were collected 15 days prior to CAR T-cell infusion (baseline) and at 14, 28, and 90 days post-infusion (D14, D28, D90). PRO included depression, anxiety, fatigue, sleep, and pain; standard assay methodology was used to assess kynurenine metabolites and cytokines (see Knight *et al.*[8] for details). Tryptophan (TRP) and metabolites — kynurenine, kynurenic acid (KA), 3-hydroxylkynurenine (3-HK), 3-hydroxyanthranilate (3-HAA), and quinolinic acid (QA) — were assessed, as were the following cytokines: interleukin 6 (IL-6), IL-8, granulocyte colony stimulating factor (G-CSF), interferon γ (IFNγ), IFNγ-induced protein 10 (IP10), fractalkine, I-309, tumor necrosis factor α (TNFα), monocyte chemoattractant protein 2 (MCP-2), and B-lymphocyte chemoattractant 1 (BCA-1). CRS grading was performed utilizing Lee 2018 criteria[10] and neurotoxicity (NTX) was graded using CTCAE version 5.0.[11] Lactate dehydrogenase (LDH) was assessed at baseline as a marker of tumor burden.

Peak cytokine and kynurenine metabolite levels were log-transformed; values below the limit of detection were replaced by half the smallest non-negative value. Mixed effects regression with random subject effect was used to analyze repeated measurements. For adjusted analyses, baseline LDH was included as a covariate in the models. All analyses were performed in R 4.0.3.[12] A two-sided 5% significance level was used.

Patient demographics, disease characteristics, and select treatment side effects are described in Table 1. Fifty-three percent of study participants reported an annual income below the Wisconsin median in 2021 of $54,660 (low SES). There was no significant difference between SES groups based on age, education level, or clinical response to therapy by D28. Given that baseline LDH was 3.4-fold higher in low *versus* high SES patients (P=0.04), we also examined SES correlations with each CAR T-cell therapy outcome variable using it as a covariate.

Most peak cytokine concentrations were significantly higher in low SES patients (Figure 1A). Low SES patients had a 15.4-fold elevation in IP-10, 7.8-fold elevation of G-CSF, 6.8-fold elevation in TNFα, 4.1-fold elevation in IL-8, and 3.4-fold elevation in I-309 and MCP-2 compared to those of high SES (all P<0.05; see the *Online Supplementary Table S1*).

3-HAA and QA were elevated in low SES patients (Figure 1B), with 3-HAA higher at D28 and QA higher at baseline, D14, and at D28 (all statistically significant; see the *Online Supplementary Table S2*). TRP, kynurenine, KA, and 3-HK did not differ between SES groups at any time points.

Patients with low SES reported a trend toward higher pain intensity on D28 and had significantly higher pain intensity by D90 (Figure 2) compared to those of high SES. Pain interference trended higher in low SES patients at baseline. Patients of low SES had consistently poor sleep quality (higher than the PSQI threshold of 5 for “poor sleep”[13]) at baseline, D28, and D90, while patients of high SES did not. Conversely, high SES patients reported significantly more days feeling fatigued on D14. Depression, anxiety, fatigue intensity, and fatigue interference were not different between SES groups (see the *Online Supplementary Table S3* for all).

There was a clinically significant difference in the proportion of patients who experienced CRS based on SES (100% of low *vs*. 57.1% of high SES patients), though this did not reach statistical significance (Table 1). Low SES patients experienced significantly higher maximum CRS and at earlier onset times compared to high SES patients. Low SES patients suffered a higher percentage of NTX, higher average NTX grade, and earlier average NTX

Table 1. Baseline patient demographics and clinical findings.

Baseline characteristic	Overall (N=15)[1]	Income range $10,001-55,0001 (N=8)[1]	Income range >$55,0011 (N=7)[1]	*P* value[2]
Age in years (range)	58 (38-72)	66.5 (46-72)	55 (38-69)	0.297
Male sex, N (%)	14 (93)	7 (88)	7 (100)	
Level of education, N (%) HS TS SC CG PGD	 2 (13) 4 (27) 3 (20) 3 (20) 3 (20)	 2 (25) 2 (25) 2 (25) 1 (12.5) 1 (12.5)	 0 (0) 2 (28.5) 1 (14.3) 2 (28.5) 2 (28.5)	0.215
Income, N (%) $10,001- 25,000 $25,001-40,000 $40,001-55,000 $55,001-70,000 $85,001-100,000 >$100,000	 2 (13) 3 (20) 3 (20) 3 (20) 2 (13) 2 (13)	 2 (25) 3 (37.5) 3 (37.5) --- --- ---	 --- --- --- 3 (42.9) 2 (28.5) 2 (28.5)	N/A
Histology, N (%) CLL DLBCL FL MCL	 2 (13) 9 (60) 1(6.7) 3 (20)	 1 (12.5) 5 (62.5) 1 (12.5) 1 (12.5)	 1 (14.3) 4 (57.1) 0 (0) 2 (28.5)	
Baseline LDH (range)	203 (121-2,074)	652 (121-2,074)	190 (147-269)	0.040
Lines of prior therapy (range)	4 (2-11)	5 (2-11)	5 (3-7)	0.678
Prior allogeneic HCT, N (%)	1 (6.7)	1 (12.5)	0 (0)	
Prior autologous HCT, N (%)	5 (6.7)	1 (12.5)	4 (57.1)	0.12
Clinical response D28, N (%) CR PD PR	 12 (80) 1 (6.7) 2 (13)	 6 (75) 1 (12.5) 1 (12.5)	 6 (85.7) 0 (0) 1 (14.3)	0.740
CRS (Yes), N (%)	12 (80)	8 (100)	4 (57.1)	0.077
Days to CRS (range)	2.5 (0.0-10.0)	2.6 (0-7)	6.8 (1-10)	0.009
Max grade CRS, N (%) 0 1 2 4	 3 (20) 7 (47) 4 (27) 1 (6.7)	 0 (0) 4 (50) 3 (37.5) 1 (12.5)	 3 (42.8) 3 (42.8) 1 (14.3) 0 (0)	0.047
NTX, N (%)	5 (33)	4 (50)	1 (14.3)	0.282
Max NTX grade, N (%) 0 1 3 4	 10 (6) 2 (13) 2 (13) 1 (6.7)	 4 (50) 1 (12.5) 2 (25) 1 (12.5)	 6 (85.7) 1 (14.7) 0 (0) 0 (0)	0.129
Days to NTX (range)	6 (0-9)	4 (0-9)	6	

CLL: chronic lymphocytic leukemia; CR: complete response; CRS: cytokine release syndrome; DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; HCT: hematopoietic cell transplantation; LDH: lactate dehydrogenase; MCL: mantle cell lymphoma; NTX: neurotoxicity; PD: progressive disease; PR: partial response; HS: high school; TS: trade school; SC: some college; CG: college graduate; PGD: post graduate degree; CRS: cytokine release syndrome; Max: maximum; NTX: neurotoxicity. Categorical variables were compared between SES groups using Fisher's exact test, Wilcoxon's rank sum test was used for ordinal and continuous measures

onset, though these did not reach statistical significance.

The association between low SES and biomarkers became insignificant when LDH was added as a covariate for some, but not all, of the cytokines (BCA-1, G-CSF, I-309, IL-6, IL-8, MCP-2, and TNFα) and one kynurenine metabolite (3-HAA). For the PRO that were worse among individuals of low SES, the associations became more pronounced/significant for pain and sleep when additionally considering LDH levels. (*Online Supplementary Tables S1* to *S3*). Among the clinical outcomes, only maximum grade of CRS became insignificant when correcting for LDH.

This study provides preliminary evidence that SES is associated with biological and clinical outcomes among patients receiving bispecific LV20.19 CAR T-cell therapy.

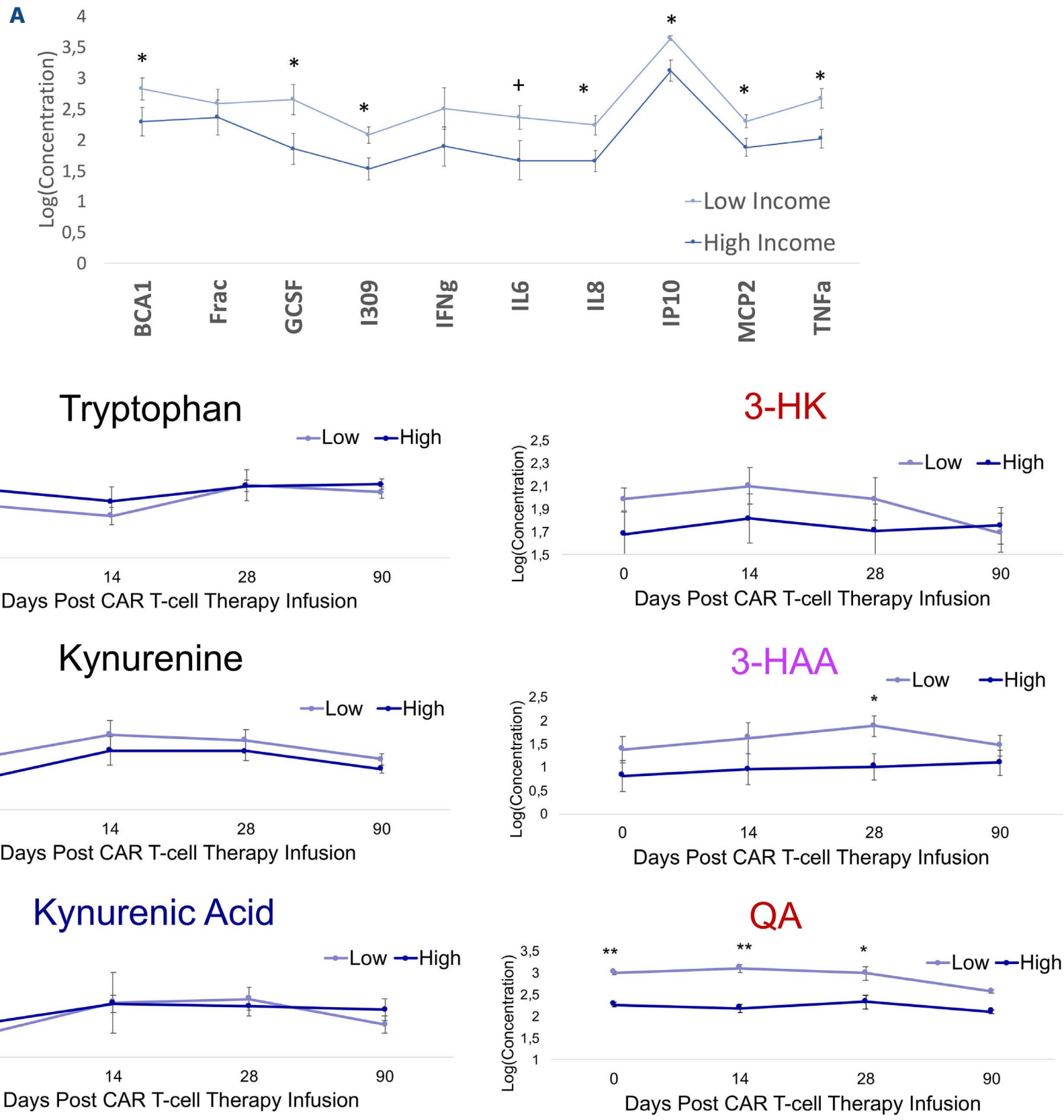

Figure 1. Peak cytokine levels and kynurenine metabolites compared between socioeconomic status groups. (A) BCA1: B-lymphocyte chemoattractant 1; Frac: fractalkine; GCSF: granulocyte colony stimulating factor (9.3-fold difference); I-309 (2.9-fold); IFNγ: interferon γ; IL6: interleukin 6; IL8: interleukin 8 (3.4-fold); IP-10: interferon γ-induced protein 10 (2.3-fold); MCP-2: monocyte chemoattractant protein 2 (2.0-fold); TNFα: tumor necrosis factor α (4.3-fold). Log transformed peak cytokine levels were compared between socioeconomic status (SES) groups using a student *t*-test. (B) Kynurenine metabolites were analyzed using linear mixed effects model and estimated marginal means (least-square means) was done to compare kynurenine metabolites between income groups at each time point. $^{+}P< 0.1$, $*P< 0.05$, $**P< 0.01$.

These pilot data demonstrate that patients of low SES have worse CAR T-cell therapy outcomes, reflected molecularly as higher baseline LDH levels, proinflammatory cytokines, and neurotoxic kynurenine metabolites, and reflected clinically as earlier CRS onset, higher CRS grade severity, and higher reported pain. Low SES patients likely presented with increased tumor burden, as represented by elevated baseline LDH.[14] While controlling for LDH tempered some of the associations between biomarkers and SES and outcomes, this was not broadly true, with LDH potentiating the SES relationship with PRO. Together, these findings support the proinflammatory and neuro-

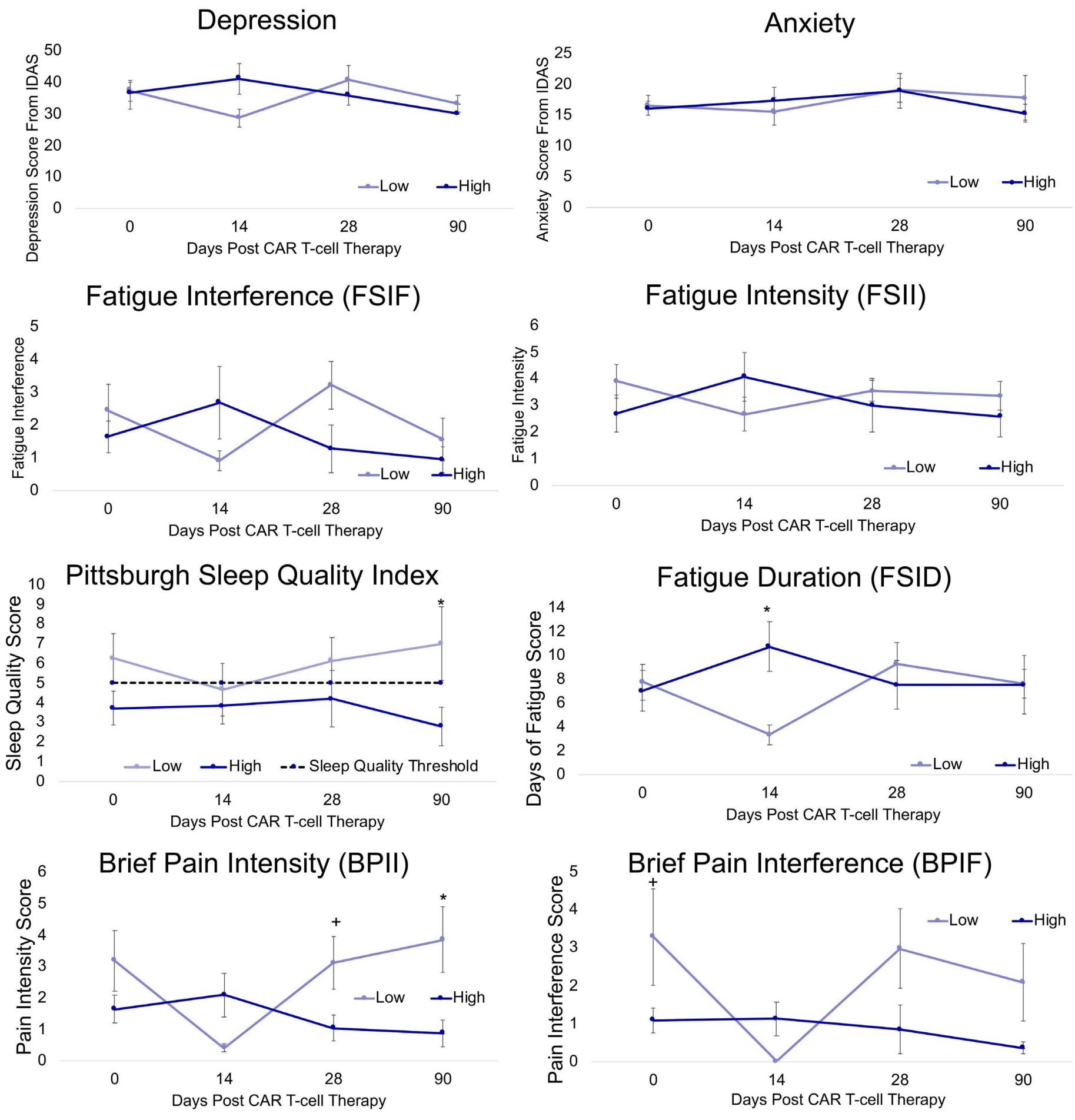

Figure 2. Patient-reported outcomes over time between socioeconomic status groups. Linear Mixed Effects Model and Estimated Marginal Means (least-square means) was done to compare kynurenine metabolites between income groups at each time point. $^{+}P< 0.1$, $*P< 0.05$, $**P< 0.01$. PRO: patient-reported outcomes; SES: socioeconomic status.

toxic metabolite priming of low SES individuals, highlighting candidate biological mechanisms in a novel cancer population that may mediate the relationship between low SES and worse cancer treatment outcomes.

The increase in proinflammatory biomarkers identified among low SES patients may also be associated with worse clinical outcomes. Many of the cytokines were additionally associated with earlier onset and worse severity of CRS. Kynurenine metabolites associated with neurotoxicity in our prior work (3-HAA and QA) were elevated among low SES patients here.[8]

This study is limited by the small sample size. These provocative findings need to be further evaluated in a larger cohort wherein additional patient-, disease-, and CAR T-related variables can be appropriately controlled for and mediational analyses conducted.

The current cohort study among individuals receiving bispecific LV20.19 CAR T-cell therapy suggests a biological impact of low SES among CAR T-cell recipients and supports the hypothesis that elevated proinflammatory cytokines and neurotoxic kynurenine metabolites are among the biological mediators of elevated risk. These novel findings identify patients of low SES as a population vulnerable to CAR T-cell therapy side effects, warranting future studies aimed at clarifying the multifactorial social impacts on biological and quality of life outcomes of cancer therapy.

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Contributions

JMK, CJH, NS and AS designed the research. JMK, NS, AS, GS and BJ performed the research. AS contributed vital new reagents or analytical tools. JMK, NS, GS and BJ coellected data. EH, JMK, CJH, AS, RW and GS analyzed and interpreted data. EH, AS, IA and RW performed statistical analysis. JMK, EH, CJH, AS, RW, NS, RNC, SWC and BJ wrote the manuscript.

Data-sharing statement

Data for this study can be attained by contacting the corresponding author.

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