CAR-T cell therapy-related cytokine release syndrome and therapeutic response is modulated by the gut microbiome in hematologic malignancies

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Immunotherapy utilizing chimeric antigen receptor T cell (CAR-T) therapy holds promise for hematologic malignancies, however, response rates and associated immune-related adverse effects widely vary among patients. Here we show, by comparing diversity and composition of the gut microbiome during different CAR-T therapeutic phases in the clinical trial ChiCTR1800017404, that the gut flora characteristically differs among patients and according to treatment stages, and might also reflect patient response to therapy in relapsed/refractory multiple myeloma (MM; n = 43), acute lymphoblastic leukemia (ALL; n = 23) and non-Hodgkin lymphoma (NHL; n = 12). We observe significant temporal differences in diversity and abundance of Bifidobacterium, Prevotella, Sutterella, and Collinsella between MM patients in complete remission (n = 24) and those in partial remission (n = 11). Furthermore, we find that patients with severe cytokine release syndrome present with higher abundance of Bifidobacterium, Leuconostoc, Stenotrophomonas, and Staphylococcus, which is reproducible in an independent cohort of 38 MM patients. This study has important implications for understanding the biological role of the microbiome in CAR-T treatment responsiveness of hematologic malignancy patients, and may guide therapeutic intervention to increase efficacy. The success rate of CAR-T cell therapy is high in blood cancers, yet individual patient characteristics might reduce therapeutic benefit. Here we show that therapeutic response in MM, ALL and NHL, and occurrence of severe cytokine release syndrome in multiple myeloma are associated with specific gut microbiome alterations.
B-cell-derived hematologic malignancies, including acute lymphoblastic leukemia (B-ALL), non-Hodgkin lymphoma (B-NHL), and multiple myeloma (MM), carry a high probability of relapse after conventional chemotherapy. With novel therapeutic strategies incorporating monoclonal antibodies, bispecific T-cell engager (BiTE) antibodies, and hematopoietic stem cell transplantation (HSCT), treatment outcomes have greatly improved. However, some patients progress to relapsed/refractory (r/r) status, with a poor prognosis. For patients with r/r B-ALL, the median overall survival (OS) is 3–6 months. The median OS is 6.2 months for patients with r/r diffuse large B-cell lymphoma (DLBCL). For r/r MM patients, the median OS is 3–9 months. There is an urgent need to explore novel treatment strategies for these malignancies.

Chimeric antigen receptor (CAR) T-cell therapy (approved by the U.S. Food and Drug Administration) recently emerged as promising for r/r B-ALL, DLBCL, and mantle cell lymphoma (MCL). In multiple myeloma, investigations targeting the B-cell maturation antigen (BCMA) yielded encouraging outcomes with reversible toxic effects such as cytokine release syndrome (CRS) and pancytopenia. However, the efficacy and toxicity have been inconsistent. No biomarker has been identified that can predict outcomes and associated toxicities after CAR-T therapy in patients.

Several studies have reported that the differences in diversity and composition of the gut microbiome might influence cancer immunotherapy response. After analyzing fecal samples from 43 melanoma patients treated with anti-programmed cell death 1 protein (PD-1) immunotherapy, significantly higher alpha diversity and abundance of Clostridiales/Ruminococaceae were found in responders, whereas Bacteroidales were significantly enriched in non-responders. In hematologic malignancies, intestinal bacteria also modulate the risk of graft-versus-host disease (GVHD) and infection after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Greater bacterial diversity and abundance of the genus Blautia were associated with reduced GVHD-related death and improved OS. However, no study has shown a potential role for the intestinal microbiota in the efficacy and toxicity of CAR-T therapy for B-cell malignancies.

Here we show the intestinal microbiome changes in patients with r/r B-cell-derived hematologic malignancies undergoing CAR-T cell treatment and investigating associations of the microbiota with clinical responses and CRS severity. Further, the potential of the gut microbiome to predict treatment outcomes and CRS severity was also explored. Our results indicated that CAR-T cell therapy-related cytokine release syndrome and therapeutic response was modulated by the gut microbiome in hematologic malignancies. These findings highlight the role of gut microbiome in CAR-T therapy.

**Results**

**Clinical trial outcomes**

Previously we reported the safety and efficacy of interim results of the trial (61 patients). Here after completion of the trial, 99 patients with relapsed/refractory multiple myeloma (r/r MM) were included (Fig. 1a). The primary outcome was to evaluate the safety of BCMA CAR-T cells in the treatment of r/r MM. All patients were evaluated for safety analysis. Cytokine release syndrome (CRS) was observed in 97% (96/99) patients, including 50 (52.1%) patients with grades 1–2 CRS, 42 (43.8%) and 4 (4.1%) with grades 3 and 4 CRS, respectively. BCMA CAR-T cells were fully controlled and managed for all patients. Of these patients, 24 died: one from sepsis caused by *Pseudomonas aeruginosa* and the other from intracranial hemorrhage (Fig. 1d). The BCMA CAR-T/CD3 T-cell percentages in peripheral blood (PB) and serum concentrations of interleukin (IL)–10 increased during CRS and differed significantly in the CR and PR groups (Fig. 1e). Patients’ temperature and C-reactive protein (CRP), ferritin, and lactate dehydrogenase (LDH) concentrations were elevated, and IL-6 and IFN-γ concentrations were significantly different in grade 3 vs grade 1 CRS (Fig. 1f and Supplementary Fig. 1a–c). The serum immunoglobulins (IgG, IgA) and immunoglobulin κ and λ light chain concentrations decreased dramatically after CAR-T (Supplementary Fig. 1d–f). Figure 1g shows the differences of positron emission tomography–computed tomography (PET-CT) scans and plasma cells detected by Wright’s stain of a bone marrow smear (43.5% vs. 0), as well as flow cytometry (68.9% vs. 0) of bone marrow cells before and after CAR-T infusion for a representative subject.

**Patient samples included for gut microbiome analysis**

Microbiome samples were not available from 12 patients and 165 sequencing depth was not sufficient for analysis on 6 patients. Finally, a total of 81 patients with r/r MM was included for gut microbiome analysis, which included 43 patients for experiment group and 38 patients for validation group (Fig. 1a). Number of samples collected, and sequencing depth were summarized in Supplementary Table 1 and Supplementary Data 3. The median age of the MM patients was 59 (range 39–75 years), and 55.8% were male (Table 1). The median number of prior lines of therapy was 4 (range 2–8), with all receiving proteasome inhibitor therapy and 53.3% immunomodulatory agents. At enrollment, 39.5% had received autologous stem cell transplantation, and 55.8% had extramedullary disease.

Three months after infusion of a median dose of 4.4 × 10^6/kg (range 1.2–6.9 × 10^6/kg) of BCMA CAR-T cells, 55.8%, 14%, and 25.5% of patients had a CR, VGPR, or PR, respectively. All 43 MM patients showed CRS, grade 1 in 8 patients (18.6%), grade 2 in 16 (37.2%), and grade 3 in 19 (44.2%). No higher grade was observed (Fig. 1d). The CRS was fully controlled and managed for all patients. Of these patients, 24 received only supportive care, 6 received supportive care plus tocilizumab treatment (IL-6 receptor-blocking monoclonal antibody), 10 received supportive care and corticosteroid treatment, and 3 received supportive care accompanied with tocilizumab and corticosteroids treatment. The antibiotics used before or during treatment were β-lactam (41 patients), Carbapenems (26 patients), Quinolone (26 patients), Aminoglycosides (1 patient), Macrolide (1 patient), Tetracyclines (4 patients), Cephalosporins (3 patients), and Glycopeptides (6 patients). Although we included age, gender, number of prior lines of therapy, CAR-T cell dose, autologous stem cell transplantation, anti-biotic use before or during treatment as covariates in our analyses, no significant differences were observed among different efficacy groups or CRS grade groups (Supplementary Tables 1–2). Two patients died: one from sepsis caused by *Pseudomonas aeruginosa* and the other from intracranial hemorrhage (Fig. 1d). Both the BCMA CAR-T/CD3 T-cell percentages in peripheral blood (PB) and serum concentrations of interleukin (IL)–10 increased during CRS and differed significantly in the CR and PR groups (Fig. 1e). Patients’ temperature and C-reactive protein (CRP), ferritin, and lactate dehydrogenase (LDH) concentrations were elevated, and IL-6 and IFN-γ concentrations were significantly different in grade 3 vs grade 1 CRS (Fig. 1f and Supplementary Fig. 1a–c). The serum immunoglobulins (IgG, IgA) and immunoglobulin κ and λ light chain concentrations decreased dramatically after CAR-T (Supplementary Fig. 1d–f). Figure 1g shows the differences of positron emission tomography–computed tomography (PET-CT) scans and plasma cells detected by Wright’s stain of a bone marrow smear (43.5% vs. 0), as well as flow cytometry (68.9% vs. 0) of bone marrow cells before and after CAR-T infusion for a representative subject.

**Changes in the intestinal microbiome during CAR-T cell therapy**

To detect changes in the gut microbiota during CAR-T therapy, we collected fecal samples from each patient at five times (F0, F1, F2, F3, and F4; Fig. 1c), where F0 denotes the baseline before chemotherapy; F1 after chemotherapy; F2 after CAR-T cell infusion.

**Phylogenetic Network Analysis**

The intestinal microbiome compositions show considerable differences in patients with r/r MM compared to healthy controls (HCs), with a very high abundance of *Blautia* and significant differences were observed among different CR, VGPR, or PR groups (Supplementary Tables 1 and Supplementary Data 3). The BCMA CAR-T/CD3 T-cell percentages in peripheral blood (PB) and serum concentrations of interleukin (IL)–10 increased during CRS and differed significantly in the CR and PR groups (Fig. 1e). Patients’ temperature and C-reactive protein (CRP), ferritin, and lactate dehydrogenase (LDH) concentrations were elevated, and IL-6 and IFN-γ concentrations were significantly different in grade 3 vs grade 1 CRS (Fig. 1f and Supplementary Fig. 1a–c). The serum immunoglobulins (IgG, IgA) and immunoglobulin κ and λ light chain concentrations decreased dramatically after CAR-T (Supplementary Fig. 1d–f). Figure 1g shows the differences of positron emission tomography–computed tomography (PET-CT) scans and plasma cells detected by Wright’s stain of a bone marrow smear (43.5% vs. 0), as well as flow cytometry (68.9% vs. 0) of bone marrow cells before and after CAR-T infusion for a representative subject.
but before the onset of CRS; and CRSb and CRSc denote the peak and during the recovery phase of CRS, respectively. The median date of FCa was 4 days (range 2–7) before CAR-T cell infusion in MM patients, the median date of FCb was 0 days (range 0–7) before CAR-T cell infusion, and the median dates of CRSa, CRSb, CRSc after CAR-T cell infusion were 2 days (range 1–5.3), 6 days (range 2.5–17.4), and 14 days (range 8–37.5), respectively.

We first evaluated the diversity of the gut microbiota in all subjects during CAR-T cell therapy in MM patients. Compared with early stage, there was a significant decrease in diversity (measured by the Shannon index) after the CAR-T therapy (Fig. 2a). This decrease was observed in the microbiome of patients receiving CAR-T therapy for r/r ALL (Supplementary Fig. 4a) or r/r NHL (Supplementary Fig. 4b). Refer to Supplementary Table 3 for details on the characteristics of r/r B-ALL...
In MM patients, notable differences in microbial alpha and within-sample diversity were observed in patients with CR and PR at CRSB stage (Fig. 3a, b). Although no differences were detected at baseline, PR patients descended more dramatically in alpha diversity and had significantly lower Shannon indices than CR patients after CAR-T infusion (Fig. 3a). As the degree of differences between CR and PR groups changed across therapeutic stages, we characterized the periods with greater differences by summarizing the amount of CR/PR-enriched OTU at each timepoint. The most pronounced differences occurred at CRSb (Fig. 3c). To explore longitudinal differences between CR and PR across all therapeutic stages, we identified OTU features with differential dynamic profiles by applying negative binomial regression-based time-course differential analysis with the maSigPro package. In total, 125 OTUs were found to have differential time-course patterns between CR and PR patients (Fig. 3d and Supplementary Data 5). The significant OTUs were further grouped into three clusters according to profiles of their abundance. Most of these OTUs were in clusters 1 and 2 (Fig. 3e). Cluster 1, characterized by enrichment in the CR group, was comprised mainly of Firmicutes increased but that of Bacteroidetes decreased at later stages compared with the baseline (Wilcoxon rank-sum test, p < 0.05, Supplementary Fig. 4d). By applying the longitudinal analysis in the Qiime2 microbiome analysis platform, we detected changes in the gut microbial communities at taxonomic levels from phylum to genus (Fig. 2d and Supplementary Data 3). We further employed a negative binominal (NB) regression model-based time-course analysis to identify genera with significant temporal changes (Supplementary Data 4). Five genera were detected by both Qiime2 and maSigPro procedures, which included increases in Enterococcus, Lactobacillus, and Actinomyces and decreases in Bifidobacterium and Lachnospira (bolder genera in Fig. 2d). Most changes were aggravated during the late stages (Supplementary Fig. 4e). Additionally, for repeated measure data (Subjects = 10), we applied Friedman’s test and found nine genera affected significantly by CAR-T therapy among which the genus Enterococcus had the largest difference between stages (Fig. 2e).

Moreover, by checking changes in the five genera in ALL and NHL patients, we observed consistent shift trends in NHL (two genera; Supplementary Fig. 4f) and ALL (four genera; Supplementary Fig. 4g), respectively. These results were further verified in another independent MM sample, showing that CAR-T therapy correlated significantly with decreased Shannon diversity (Supplementary Fig. 4c) and increased abundance of genus Enterococcus and Actinomyces (Supplementary Fig. 4h).

**Table 1** Baseline characteristics of MM patients and validation MM patient samples included in final fecal microbiome analysis

|                     | Total N = 43 (%) | Validation sample N = 38 (%) |
|---------------------|-----------------|-----------------------------|
| **Age**             |                 |                             |
| Median              | 59              | 60                          |
| Range               | 39–75           | 16–74                       |
| **Gender**          |                 |                             |
| Male                | 24 (55.8)       | 17 (44.7)                   |
| Female              | 19 (44.2)       | 21 (55.3)                   |
| **Number of prior lines of therapy** | | |
| Median              | 4               | 4                           |
| Range               | 2–8             | 2–7                         |
| **CAR-T cell dose (×10^6/kg)** | | |
| Median              | 4.4             | 2.1                         |
| Range               | 1.2–6.9         | 0.74–6                      |
| **Autologous stem cell transplantation** | | |
| No                  | 26 (60.5)       | 25 (65.8)                   |
| Yes                 | 17 (39.5)       | 13 (34.2)                   |
| **Extramedullary disease** | | |
| No                  | 19 (44.2)       | 17 (44.7)                   |
| Yes                 | 24 (55.8)       | 21 (55.3)                   |
| **Prior Pi therapy** |                 |                             |
| No                  | 0               | 0                           |
| Yes                 | 43 (100)        | 38 (100)                    |
| **Prior IMiD therapy** |                 |                             |
| No                  | 2 (4.7)         | 2 (5.3)                     |
| Yes                 | 41 (95.3)       | 36 (94.7)                   |

*Pi Proteasome inhibitors (Bortezomib/Carfilzomib/haxorizomib); IMiD immunomodulatory agent (Lenalidomide/Thalidomid/Pomalidomide).
of OTUs, which belong to the phyla Firmicutes and Bacteroidetes and the orders Clostridiales and Bacteroidales. Cluster 2 was comprised of OTUs from a broader taxonomy, which included the orders Clostridiales and Bacteroidales. In genus level, we identified 30 genera with differential timepoints across therapy stages. Red represents significant change in the right side denotes difference of each genus between two therapy stages. Significant p values were labeled in the boxes. Significances by two-tailed Wilcoxon rank-sum tests with FDR correction.

**Fig. 5** | Changes of microbial composition during CAR-T therapy in MM patients. 

**a** Shannon diversity indices of gut microbiome across CAR-T stages in all myeloma patients. Differential tests by Friedman’s tests and two-tailed Wilcoxon rank-sum tests for 10 pairwise comparisons of the five timepoints (n = 14). Bonferroni correction was applied for multiple testing: FDR < 0.05, **FDR < 0.01.** For FCA versus CRSc, adjusted p = 0.023; FCB versus CRSc, adjusted p = 0.009; CRSa versus CRSc, adjusted p = 0.017. Boxplots indicate the median (thick bar), first and third quartiles (lower and upper bounds of the box, respectively), lowest and highest data value within 1.5 times the interquartile range (lower and upper bounds of the whisker). 

**b** Pairwise Spearman correlation of OTU-level bacterial abundance across different timepoints. Rho value for each significant correlation is labeled inside box. 

**c** Stacked bar plot of mean phylum-level phylogenetic composition of bacterial taxa in myeloma patients across different therapy stages. 

**d** Significant features identified by longitudinal analysis in Qime2 “feature-volatility” plugin to identify taxonomic features associated with therapy stages. Scatter plot shows importance and average change of each important features by the longitudinal analysis. Genus-level features are labeled in the figure. 

**e** Bar plot in the left shows significantly changed genera across the therapy identified by Friedman’s tests (FDR < 0.05, n = 14). Effect size was estimated by Kendall’s W Test. Heatmap in the right side denotes difference of each genus between two therapy stages. Red represents significant enrichment while blue represents significant depletion of the genus in the posterior stage comprising with the anterior stage. Significant p values were labeled in the boxes. Significances by two-tailed Wilcoxon rank-sum tests with FDR correction.

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**Fig. 2** | Changes of microbial composition during CAR-T therapy in MM patients. 

**a** Shannon diversity indices of gut microbiome across CAR-T stages in all myeloma patients. Differential tests by Friedman’s tests and two-tailed Wilcoxon rank-sum tests for 10 pairwise comparisons of the five timepoints (n = 14). Bonferroni correction was applied for multiple testing: FDR < 0.05, **FDR < 0.01.** For FCA versus CRSc, adjusted p = 0.023; FCB versus CRSc, adjusted p = 0.009; CRSa versus CRSc, adjusted p = 0.017. Boxplots indicate the median (thick bar), first and third quartiles (lower and upper bounds of the box, respectively), lowest and highest data value within 1.5 times the interquartile range (lower and upper bounds of the whisker). 

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abundance of these significantly changed bacteria of interest in an independent 38 MM validation sample. We found that abundance of genera *Sutterella* and *Prevotella* were higher in CR group than that in non-CR group at multiple stages. No significance was observed for *Collinsella* and *Bifidobacterium* (Supplementary Fig. 5d).

To further demonstrate the association between these taxa and outcome, we assessed PFS following CAR-T therapy. By stratifying patients by tertile of bacterial abundance, we observed that for *Sutterella*, patients in the highest-abundance tertile had significantly prolonged PFS (Fig.4g). Even after stratification by timepoints, this association remained significant (Supplementary Fig. 6a). However, for genus *Faecalibacterium*, which was reported to be significantly associated with PFS and anti-PD-1 therapy19, we did not observe an association (Supplementary Fig. 6b, c).

**Associations between gut microbiome and CRS**

Manifestations of severe CRS, namely high fever and greater amounts of cytokines, typically develop within several days after CAR-T cell infusion and may cause death if untreated27. We scaled CRS from level 1 to 528. To analyze associations between bacterial communities associated with CRS, we compared patients with severe (level 3) versus mild (level 1) CRS and severe and moderate CRS (level 2) in MM patients. We found 146 OTUs with different time patterns in the severe and mild groups (Supplementary Fig. 7 and Supplementary Data 7), and 99 OTUs with different patterns in the severe and moderate CRS groups (Supplementary Fig. 8 and Supplementary Data 8). The profiles of the OTU clusters for the comparisons were similar, with OTUs in clusters 1 and 3 having a higher abundance during late therapy in patients with severe versus mild CRS (Supplementary Figs. 7b and 8b).

**Fig. 3** | Association of compositional differences in gut microbiome with responses to CAR-T therapy in MM patients. a Shannon diversity indices of gut microbiome differed between CR and PR groups across CAR-T stages. Significances were assessed by two-sided Wilcoxon rank-sum test (n = 35). P values were 0.077, 0.040, 0.036 for FCh, CRSa, and CRSb, respectively. Boxplots indicate the median (thick bar), first and third quartiles (lower and upper bounds of the box, respectively), lowest and highest data value within 1.5 times the interquartile range (lower and upper bounds of the whisker). b Principal coordinate analysis of fecal samples in CRSb stage by response (CR versus PR) using Canberra distance. P value was calculated by PERMANOVA (n = 35). c Summary of number of PR or CR-enriched OTUs in different therapy stages. Difference between CR and PR groups was assessed by two-sided Wilcoxon rank-sum test. P value significant cutoff was 0.05 (n = 35). d Heatmap for abundance of OTUs with significant temporal differences between CR and PR groups identified by maSigPro (FDR < 0.05). Rows denote bacterial OTUs grouped into three sets according to regression coefficients and sorted by mean abundance within each set. Individual fecal samples were organized in columns and grouped by therapy stages. Columns in the blue and red dashed box show abundance and longitudinal changes of these OTUs in CR and PR groups across the five timepoints. Color of the heatmap is proportional to OTU abundance (red indicates higher abundance and blue indicates lower abundance). e Profiles of significant gene clusters correspond to d. Solid lines denote median profile of abundance of OTUs within cluster for each experimental group through time. Fitted curve of each group is displayed as dotted line. f Phylogenetic composition of OTUs within each cluster in d at phylum and order levels.
Correlation of gut microbial functions with CAR-T therapy

To determine if gut microbial functions correlated with CAR-T therapy, we first inferred community function of MM patients using Phylogenetic Investigation of Communities by Reconstruction of Unobserved State (PICRUSt2). By applying time-course differential analysis, we identified differential pathways related to fatty acid metabolism, glutathione metabolism, quinone biosynthesis and glycan degradation (Supplementary Fig. 10) in the MM cohort. Further, we compared several pathways associated with phosphonate and its metabolism, high CRS grade (Fig. 5a, b). In the 38 validation MM patients, no significance was observed for *Bifidobacterium* or *Leuconostoc* among different CRS grade groups (Supplementary Fig. 9).

**Correlation of gut microbial functions with CAR-T therapy**

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amino acid metabolism, lipoic acid metabolism, amino sugar, and nucleotide sugar metabolism and antibiotic synthesis (Supplementary Fig. 11).

Likewise, we performed differential analysis of PICRUSt2 predicted functions in the 38 validation MM cohort. Comparing PR with CR, differential pathways concerning glutamate (D-Glutamine and D-glutamate metabolism), glycan (Glycan biosynthesis and metabolism), arginine, proline (D-Arginine and D-ornithine metabolism, Arginine and proline metabolism) and phenylalanine (phenylpropanoid biosynthesis) were revealed (Supplementary Fig. 12a), among which the pathways related to glutamate and phenylalanine metabolism were endorsed in differential analysis of predicted KEGG pathways between PR and CR groups in the discovery MM sample (Supplementary Fig. 10a). Lipopolysaccharide and steroid biosynthesis pathways were also consistently found to be differ between the CR and PR group by differential analysis of predicted pathways (Supplementary Fig. 10a) and metabolites (Supplementary Fig. 12a). Referring to the CRS grade-related pathway, difference in glycerolipid metabolism pathway was...
reproducible detected in both the discovery (Supplementary Fig. 11a) and validation MM samples (Supplementary Fig. 12c).

In addition, we applied metabolic Liquid Chromatography Mass Spectrometry (LC-MS) to quantify concentration of fecal metabolites during CRS. Intermediates (Choline, \( \epsilon \)-Cysteine, S-Sulf Hy-L-cysteine, Rosmarinic acid, \( \epsilon \)-Phenylalanine, and 2-Phenylacetamide) involved in multiple amino acid metabolism pathways were differentially abundant between CP and CR group when during CRS (\( p \)-value = 0.05). We also identified metabolites concerning phosphate and phosphonate metabolism (Bialaphos) and steroid biosynthesis (Desoxycortone) to be differ between CR and PR (Supplementary Fig. 13). In differential analysis between CRS groups, we identified phosphocreatine which annotated to arginine and proline metabolism (Supplementary Fig. 14). Moreover, three abovementioned pathways (i.e., tyrosine metabolism, phenylalanine metabolism, phosphate, and phosphonate metabolism) were also indicated to have differentially abundances between the CR and PR group in the predicted pathway analysis (Supplementary Fig. 10). Two pathways (tyrosine metabolism and phenylalanine metabolism) were also differed among patients with different CRS grades (Supplementary Fig. 11). Additionally, we performed pathway enrichment analysis of differentially abundant metabolites between the CR and PR subjects to reveal distinct on metabolic functions (Supplementary Fig. 15a). Two pathways (Phenylalanine, tyrosine and tryptophan biosynthesis; Riboflavin metabolism) reached marginal significance (\( p = 0.07 \)). These concordant findings strengthened the results of functional prediction analysis and highlighted the importance of amino acid metabolism during the CAR-T therapy.

Correlation of CRS-related cytokines with gut microbes
Primary inflammatory markers of CRS are cytokines, such as IL-6, IL-2, IL-10, interferon gamma (IFN-\( \gamma \)), and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)). Various cytokines are elevated in the serum of patients experiencing CRS after CAR-T cell infusion\(^{35}\). By assessing serum cytokine concentrations and immune cell numbers during CAR-T, we observed significantly increased amounts of serum inflammatory cytokines (IL-6, CRP, IFN-\( \gamma \), D-dimer, ferritin) but low numbers of immune cells (monocytes, lymphocytes, neutrophils, leukocytes) in severe CRS (Fig. 5c). We also compared serum cytokine concentrations and immune cell numbers in CR and PR, observing significant differences for many of them (see Supplementary Fig. 16).

To explore further associations between the gut microbiome and CRS during CAR-T therapy, we determined whether serum cytokine concentrations and numbers of PB immune cells correlated with the abundance of gut microorganisms (Fig. 5d). By assessing common within-individual correlation for repeated measures\(^{36}\), we constructed correlation network between gut microbes, cytokines, and immune cells (Fig. 5c). The top significant correlation pairs were MCP-1 and Lactobacillus, lymphocyte and Clostridium, IL-15 and Lactobacillus, leukocyte and Veillonella (Fig. 5d). In addition, serum level of lymphocyte was negatively correlated with IL1 genera, including multiple genera related to CRS level such as Bifidobacterium, Butyrivibocida and Oscillospira. M1 and M2 macrophages, which play a key role in CRS initiation, did not show significant correlation with any microbes.

Discussion
Although several studies have revealed the critical role of the gut microbiome in treatment responses and survival after administration of another important immunotherapy — immune checkpoint inhibitor (e.g., PD-1, PD-L1) therapy\(^{37}\), no study has reported on the association between the gut microbiome and CAR-T therapy. In this study, we describe the changes of the gut microbiome during CAR-T therapy and associations with treatment responses and CRS severity in CAR-T treated patients with B-cell malignancies. Although neurotoxicity is another major toxicity associated with CAR-T cell therapy, we were not able to analyze the microbiome in relation to neurotoxicity incidence and severity because of very limited patients presented in the MM patients. Future work with larger number of patients is needed in order to explore the relationship between microbiome and neurotoxicity after CAR-T cell treatment.

Some of the bacterial genera with differences in abundance in CR versus PR patients have been reported to be involved in the regulation of the immune response, including to immunotherapy. Faecibacterium, related to enhance antitumor immune responses and survival after anti-PD-1 therapy in melanoma\(^{38,39}\), was in this study associated with CR. Multiple species within the genera Bifidobacterium and Collinsella increased in responders to anti-PD-1 therapy for melanoma\(^{40}\), resulting in depleted peripherally derived colonic regulatory T cells, increased Baf3-lineage dendritic cells (DC), and augmented T-helper 1 (Th1) responses and thus better immune-mediated tumor control\(^{41}\). Here, we observed an increased abundance of these two bacteria in CR patients, suggesting a similar response-associated effect of these taxa on the immune system across cancer types and therapeutic strategies. Moreover, in a recent study, taxonomic analysis by 16 S sequencing of the fecal microbiome showed that members of Ruminococcus, Faecibacterium, and Bacteroides were associated with response to CD19 CAR-T cell therapy. Higher abundances of microbial taxa with the class Clostridia, including the genera Ruminococcus, Faecibacterium were associated with day 100 complete response and no toxicities in patients with ALL and NHL after receiving CD19 CAR-T cell treatment were observed\(^{42}\).

Nevertheless, some taxa might have effects that are specific for cancer or therapy types. For example, high abundance of genus Sutterella was associated with both CR and prolonged survival after CAR-T therapy. However, previous studies reported higher numbers of Sutterella in non-responders versus responders in non-small-cell lung cancer (NSCLC) treated with nivolumab\(^{43}\). Besides, in this study, we observed contradictory results for the genus Bifidobacterium, Roseburia, and Collinsella in three types of hematologic malignancy (Supplementary Fig. 2). This indicates a potentially distinct involvement or function of some bacteria in different cancer types and treatments. But these findings require confirmation in studies with larger cohorts.

Gut microbial communities contribute to inter-individual variation in cytokine responses\(^{44}\). In this study, Bifidobacterium and Leuconostoc were enriched in myeloma patients with severe CRS while Butyricicoccus were enriched in patients with mild CRS. Bifidobacterium is a commonly used probiotics to enhance host immune. Another report demonstrated that Bifidobacterium correlated with the production of multiple cytokines (e.g., IFN-\( \gamma \)) in a stimulus-specific pattern\(^{45}\). Other oral supplementation tests concluded that Bifidobacterium could enhance nonspecific cellular immune response by activation of immune cells and release of various cytokines\(^{46}\). Moreover, it is worth noting that species composition of the Bifidobacterium flora will affect cytokine production. Cultivation of murine macrophage-like cells with divergent Bifidobacterium strains differentially stimulated production of proinflammatory TNF-\( \alpha \), IL-12, and IL-6\(^{47}\). Thus, we proposed that the increased Bifidobacterium abundance in patients with server CRS may compose of more diverse Bifidobacterium strains predisposing toward CRS in CAR-T therapy. Further studies should pay attention to species and strain level composition of Bifidobacterium and their association with CRS. Besides, all patients with severe CRS in this study were treated with tocilizumab and/or corticosteroid. In future work, we will apply the CRS mice model to investigate the effects of Tocilizumab and corticosteroid on gut microbiome after CAR-T cell treatment.

The mechanisms through which gut microbes modulate host immunity are largely unknown. Gut microbial communities modulate host defenses mainly through the release of intermediary metabolites rather than by direct interaction between specific microorganisms and immune cells\(^{48}\). Multiple bioactive gastrointestinal metabolites produced by gut microbes, such as amino acids, short-chain fatty acids
(SCFAs; e.g., butyrate), and bile acids, exert immunomodulatory functions through immune cell metabolic reprogramming or transcriptional and epigenetic modulation of immune-related genes\(^{39}\). Lipopolysaccharide (LPS) from some pathogens is a well-known endotoxin that can stimulate the release of a variety of cytokines/chemokines\(^{40,41}\). Peptidoglycans in bacterial cell walls are a conserved PAMP that trigger innate inflammatory responses throughout the body\(^{42}\).

By comparing community functions inferred from PICRUSt2, we found pathways concerning amino acid metabolisms to have differentially abundances in samples with distinct treatment outcomes and CRS grades in myeloma patients. Phenylalanine metabolism pathways were suggested to be differentially abundant between CR and PR patients in both discovery and validation MM patients through differential analysis of predicted functions and metabolites. Differential analysis of LC-MS-based fecal metabolites revealed that intermediate metabolites of phenylalanine metabolism, L-Phenylalanine and 2-Phenylacetamide, were all enriched in the CR versus PR patients. The roles and underlying mechanisms of intestinal phenylalanine in regulating immunotherapies worth further investigating. Amino acids, such as cysteine, glutamine, phenylalanine, tryptophan, and arginine, play an important role in regulating immune responses by activating T lymphocytes, B lymphocytes, natural killer cells and macrophages, regulating cellular redox state, gene expression and lymphocyte proliferation, and stimulating production of antibodies, cytokines, and other cytokotoxic substances\(^{43,44}\). Gut microbiota is an essential mediator that keeps homeostasis of host amino acids. In the intestine, gut microbes help to metabolize amino acids from food or synthesize several essential amino acids de novo and, in turn, regulate innate and adaptive immunity of hosts\(^{45}\). Compositional difference of gut microbiota may thus lead to disparate clinical outcomes and CRS for the CAR-T therapy. However, arginine and tryptophan metabolism, which correlated with CRS in COVID-19\(^{46}\), were not identified in this study. This might be due to technical limitation of amplicon sequencing and function inference of PICRUSt2. Metagenomic sequencing is thus recommended to further explore association of bacterial functions with CAR-T therapy.

In this report, we observed significant correlation of gut microbiome with treatment outcome and CRS grade of CAR-T therapy. To further demonstrate the correlation and investigate underlying mechanisms, more wet-lab experiments are needed in future. Germ-free mice are helpful tools to validate function of gut microbiome. For example, fecal microbiota transplantation (FMT) from CR and PR patients into germ-free mice to construct mouse colonized by donor microbiota. Then physiological and biochemical response of these FMT-treated mice to myeloma cells and CAR-T cells could be surveyed. Moreover, gut microbiota could be damaged by antibiotic treatment and rescued by FMT, which could help to validate the function of gut microbiome in immunotherapy. On the other hand, genus Sutterella was found to be important biomarkers for treatment outcome. Further study should demonstrate the predictive role of Sutterella in a larger cohort of myeloma and explore its predictive capacity in CAR-T therapy of other types of tumors. To study correlation of genus Bildobacterium with CRS grade, probiotic supplement of Bildobacterium species to myeloma or CAR-T mouse model could help to reveal immune responses caused by Bildobacterium. Other oral supplement strategies include bacterial metabolites, such as amino acids, fatty acids, cytokotoxin, could also be used to demonstrate mechanism underlying the effect of gut microbes on the CAR-T therapy.

In addition to myeloma, CAR-T therapy has been applied to other blood cancers and solid tumors. The link between the gut microbiome and different cancer types needs to be studied systematically. Our research describes associations between changes in the gut microbiome of CAR-T patients and clinical responses and survival. This will open an avenue for investigating the interaction of the gut microbiome and CAR-T cells and lead to novel ways to improve the therapeutic efficacy of CAR-T therapy by targeting the gut microbiome.

As one of the most prominent treatment strategies for hematologic malignancies, CAR-T cell therapy has recently received great attention. Here for the first time, we found that the dynamic changes in the gut microbiome were related significantly with therapeutic response and CRS during CAR-T treatment of hematologic malignancies (B-ALL, B-NHL, and MM). These findings will aid the development of novel biomarkers for predicting treatment outcome and CRS severity, thereby optimizing the management of these patients while reducing potential toxicities.

**Methods**

**Study design and protocol**

The study was approved by the Institutional Review Board of the First Affiliated Hospital, School of Medicine, Zhejiang University and was registered in the Chinese Clinical Trial Registry (ChiCTRIS00017404). All patients provided written informed consent for participation in accordance with the guidelines of the Declaration of Helsinki and signed agreement for collection and analysis of microbiome samples. Patient inclusion criteria were: (1) age <75 years; (2) relapsed or refractory BCMA-positive MM before CAR-T cell treatment; and (3) expected survival >12 weeks and adequate performance status and organ function to tolerate treatment. Exclusion criteria were: (1) pregnancy or lactation; (2) having received systemic (except inhaled) steroids in the previous 2 weeks or gene therapies; (3) having medical conditions such as severe mental illness, clinically significant cardiovascular disease, severe renal or hepatic dysfunction, or active infection; and (4) any conditions that might increase treatment risks. Data and sample collection were carried from 1 July 2018 to 30 September 2021. Patient information and the methods related to two types of cancer (ALL and NHL) are presented in the Supplementary Materials.

Peripheral blood mononuclear cells (PBMCs) were obtained from each patient by leukapheresis for CAR-T cell preparation. The purified CD3\(^+\) T cells were transduced with lentiviral vector to express BCMA CAR (Fig. 1b). Then the engineered T cells were expanded ex vivo under interleukin-2 stimulation. All patients received lymphodepletion with fludarabine (30 mg/m\(^2\) of body surface area daily on days −4, −3, and −2) and cyclophosphamide (500 mg/m\(^2\) daily on days −3 and −2) followed by an infusion of BCMA CAR-T cells on day 0\(^{11}\). The primary response outcome, defined by the guidelines from the International Myeloma Working Group (IMWG) as a complete response (CR), very good partial response (VGPR), or partial response (PR) in the third month after CAR-T treatment\(^{12,44}\). CRS was graded by the Lee criteria\(^{6}\).

**Microbiome sample collection and restoration**

Gut microbiome samples were collected at five timepoints (Fig. 1c). All fecal samples were collected with the GUHE Flora Storage kit (Zhejiang Hangzhou Equipment Preparation 201906S2, GUHE Laboratories, Hangzhou, China), which maintains microbial DNA stability at room temperature for as long as 1 month. All samples were frozen at −80°C prior to DNA extraction. The stages of FCA, FCB, and CRSa were defined as early stages and CRSb and CRSc as late stages. The CRS grade 1 was defined as Mild, CRS grade ≤2 as Moderate, and CRS grade ≥3 as Severe.

**Assessment of serum cytokine concentrations**

All blood samples were stored at 4°C until centrifugation at 5000 rpm for 6 min. The supernatant liquids were quantified with the BD Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit and its corresponding software (BD Biosciences) according to the manufacturer’s instructions. Plasma levels of MIP-1α, GM-CSF, MCP-1, IL-15, IL-1β, IL-1α,
and IL-17α were determined by the Bio-Rad human Multi-cytokine detection array.

**Assessment of CAR-T cell expansion and persistence**

Serial PB samples were collected in BD Vacutainer K$_2$EDTA tubes (BD Biosciences) before and after CAR-T cell infusion. The expansion of CAR-T cells in vivo was determined by detecting the CAR-T ratio continuously in PB as described$^{49, 50}$. BCMA CAR-T expression was assessed using biotin-SP-conjugated F(ab′)2 fragment goat anti-mouse IgG, F(ab′)2 fragment-specific antibody, and the secondary staining reagent streptavidin-FITC (BioLegend, 405202) or streptavidin-PE (BioLegend, 405204) using a dilution of 1:50. The flow cytometry gating strategy is presented in Supplementary Fig. 17.

**DNA extraction**

Total bacterial genomic DNA samples were extracted using the MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). The quantity and quality of extracted DNA was assessed using both the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis.

**Bacterial 16S rRNA gene sequencing**

The V4 region of the 16S rRNA gene was amplified with a bacterial universal primers: 515F (5′-GTCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAAT-3′). The primers used for amplification contain adapters for the HiSeq platform and single-end barcodes allowing pooling and demultiplexing sequences of PCR products. Amplified sequences were purified with AMPure XP beads (Agencourt, Inc, Beverly, Manchester, MA, USA) and AxyPrep DNA Gel Extraction Kit (Axygen, Inc, Union City, CA). Qualified PCR products were sequenced with the HiSeq platform (Illumina, Inc. San Diego, CA, USA) using the 2 × 150-bp paired-end sequencing protocol.

**Amplicon data processing**

Sequenced reads were demultiplexed according to barcodes. Paired-end reads were merged with the fastq-mergepairs command from VSEARCH (v. 2.4.4)$^{31}$. The minimum length of overlap between paired-end reads was set to 5. Merged reads were then imported into Qime2 (v. 2020.2)$^{32}$. Jointed reads were processed by the qime quality-filter q-score-join command to filter sequences with low-quality scores. Sequences were denoised with the Deblur workflow$^{33}$. Amplicon sequence variants (ASVs) were summarized with the feature-table summarize command. To calculate phylogenetic diversity, a rooted phylogenetic tree was constructed using the align-to-tree-mafft-fas-ttree pipeline from the q2-phylogeny plugin within Qime2. The pipeline performed a multiple sequence alignment of the ASV sequences and then masked the alignment to remove positions that are highly variable. The masked alignment was used to generate a phylogenetic tree by FastTree program$^{34}$. Alpha and beta diversity matrices were generated through the q2-diversity plugin. Non-parametric tests were performed by the least-squared technique and the second step creates best regression models for each feature by using stepwise regression to identify features with different profiles between experimental groups. We used as input, the normalized relative abundance per sample. Gene family abundance per sample was summarized and grouped into KEGG orthologs (KOs). To facilitate the interpretation of functional results, KOs were further summarized into KEGG pathways on the basis of structured pathway mappings. For differential pathway analysis, we applied the two-sided Welch’s t test to identify discriminative KEGG pathways concerning clinical responses (PR versus CR) and CRS level (level 1 versus level 3).

**Bioinformatics and statistical analysis**

Comparisons of alpha and taxonomic abundances between two groups were conducted with the Wilcoxon rank-sum test, while comparisons among three or more groups were conducted using the Kruskal-Wallis rank-sum test. For beta diversity analysis, a PCoA plot was generated with weighted Unifrac distances. To test the significance of between-sample diversity alternation, permutational analysis of variance (PERMANOVA) was performed with the adonis function within the R (v. 3.6.2) package vegran (v.2.5-6). Clinical data were analyzed using SPSS software (v. 23.0). Flow cytometry data were analyzed using FlowJo 10 software.

For time-course differential analysis, the R package maSigPro (v.1.58.0)$^{35, 36}$ was used to find taxonomic features with significant temporal changes and significant differences between experimental groups (e.g., clinical response and CRS grade groups). Specifically, the maSigPro algorithm defined a generalized regressive model by dummy variables followed by two regression steps: the first one selects features with non-flat profiles by the least-squared technique and the second step creates best regression models for each feature by using stepwise regression to identify features with different profiles between experimental groups. We used as input, the normalized relative abundance (scaled to 100 million) and excluded features that did not occur in more than 90% of all samples. We employed a negative binomial regressive model for the microbial counts data and ran maSigPro on therapy stages with a degree of 4. All features with a significant group difference were exported. The significant features were further clustered together using the hclust function (Stats package v.3.6.2) method according to the patterns of their relative abundance. For each cluster, a median profile and fitted curve of all
included features were summarized to visualize the profile pattern. For genera that was identified by maSigPro, we applied generalized linear-mixed models (GLMMs, Stats package v.3.6.2)) to identify genera with different abundances before and after CAR-T infusion. For multiple testing correction, FDR was applied.

The Linear Discriminant Analysis (LAD) effect size (LEfSe, v.1.1) algorithm was employed to identify differentially abundant features between groups (e.g. between clinical response and CRS grade). The method first detected features with significant differential abundance using the non-parametric factorial Kruskal–Wallis rank-sum test with pre-defined α of 0.05. Significant features were then used to build a LDA model for estimating the effect size of each differentially abundant feature. The LDA score threshold for discriminative features was set to 2.0.

To identify early predictive biomarkers with respect to clinical response (PR vs. CR), we implemented a random forest (RF) feature selection procedure within the R package caret (v. 6.0-85). The recursive feature elimination (RFE) algorithm in caret package with 5-fold cross validation was applied for feature selection. An optimized number of feature sets was determined by performance of 5-fold cross validation. To depict the receiver operating characteristic (ROC) curve and calculate the area under the curve (AUC), the pROC package (v.116.1) was utilized.

For PFS analysis, subjects were classified as high, medium, or low based on tertiles of the distribution of specific taxon abundance (e.g., genus Sutterella). Time to progression was defined as the interval (in days) from the date of CAR T-cell infusion to the date of disease progression. Survival curves were estimated using the Kaplan–Meier product-limit method and compared using the log-rank test within the R package survminer (v.0.4.7).

We applied repeated measures correlation (rmcorr, v.0.4.5) analysis to test the association between bacterial abundance and concentration of immune cells and inflammatory factors. Only genus-level features deemed to be associated with clinical response and CRS grades were included in this analysis. Associations with FDR less than 0.05 were depicted using Cytoscape (v.3.9.0).

**Reporting summary**
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The raw sequence reads used in this study have been deposited in the Sequence Read Archive (SRA) of the NCBI under accession number PRJNAS13944. All software packages used for the study are publicly available. Source data are provided with this paper.

**Code availability**
Scripts used to produce figures, alongside scripts used in data analysis are available at: https://github.com/jjlea/CART-microbiome.

**References**
1. Stephenson, R. & Singh, A. Drug discovery and therapeutic delivery for the treatment of B and T cell tumors. Adv. Drug Deliv. Rev. 114, 285–300 (2017).
2. Habermann, T. M. et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J. Clin. Oncol. 24, 3121–3127 (2006).
3. Goebeler, M. E. et al. Bispecific T-Cell Engager (BiTE) Antibody Constricts Blinatumomab for the Treatment of Patients With Relapsed/Refractory Non-Hodgkin Lymphoma: Final Results From a Phase I Study. J. Clin. Oncol. 34, 1104–1111 (2016).
4. Lokhorst, H. M. et al. Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. N. Engl. J. Med. 373, 1207–1219 (2015).
5. Sonneveld, P. Management of multiple myeloma in the relapsed/refractory patient. Hematol. Am. Soc. Hematol. Educ. Program 2017, 508–517 (2017).
6. Gokbuget, N. et al. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. Blood 120, 2032–2041 (2012).
7. Kantarjian, H. M. et al. Defining the course and prognosis of adults with acute lymphoblastic leukemia in first salvage after induction failure or short first remission duration. Cancer 116, 5568–5574 (2010).
8. Crump, M. et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood 130, 1800–1808 (2017).
9. Kumar, S. K. et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. Leukemia 26, 149–157 (2012).
10. Maude, S. L. et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N. Engl. J. Med 371, 1507–1517 (2014).
11. Maude, S. L. et al. Tisagenlecleucel in children and young adults with B-Cell lymphoblastic leukemia. N. Engl. J. Med. 378, 439–448 (2018).
12. Neelapu, S. S. et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N. Engl. J. Med. 377, 2531–2544 (2017).
13. Zhao, W. H. et al. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. J. Hematol. Oncol. 11, 141 (2018).
14. Raje, N. et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N. Engl. J. Med. 380, 1726–1737 (2019).
15. Brudno, J. N. et al. T cells genetically modified to express an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. J. Clin. Oncol. 36, 2267–2270 (2018).
16. Xu, J. et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen of chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. J. Clin. Oncol. 36, 2267–2270 (2018).
17. Yi, M. et al. Gut microbiome modulates efficacy of immune checkpoint inhibitors. J. Hematol. Oncol. 11, 47 (2018).
18. Vetizou, M. et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 350, 1079–1084 (2015).
19. Vitetta, E. et al. The intestinal microbiota in allogeneic hematopoietic cell transplant and graft-versus-host disease. Blood 128, 1688–1700 (2016).
20. Zitvogel, L., Ma, Y., Raoul, D., Kroemer, G. & Gajewski, T. F. The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. Science 359, 1366–1370 (2018).
21. Gopalakrishnan, V. et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 359, 97–103 (2018).
22. Yi, M. et al. Gut microbiome modulates efficacy of immune checkpoint inhibitors. J. Hematol. Oncol. 11, 47 (2018).
23. Staffas, A., Burgos da Silva, M. & van den Brink, M. R. M. The intestinal microbiota in allogeneic hematopoietic cell transplant and graft-versus-host disease. Blood 128, 927–933 (2017). Blood 129, 2204 (2017).
24. Jenq, R. R. et al. Intestinal blautia is associated with reduced death from graft-versus-host disease. Biol. Blood Marrow Transplant. 21, 1373–1383 (2015).
25. Zhang, M. et al. Risk factors associated with durable progression-free survival in patients with relapsed or refractory multiple myeloma treated with anti-BCMA CAR T-cell therapy. Clin. Cancer Res. 27, 6384–6392 (2021).
25. Segata, N. et al. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60 (2011).
26. Oh, T. G. et al. A universal gut-microbiome-derived signature predicts cirrhosis. Cell Metab. 32, 878–888.e876 (2020).
27. Norelli, M. et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. Nat. Med. 24, 739–746 (2018).
28. Lee, D. W. et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124, 188–195 (2014).
29. Brudno, J. N. & Kochenderfer, J. N. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood 127, 3321–3330 (2016).
30. Bakdash, J. Z. & Marusich, L. R. Repeated measures correlation. Front. Psychol. 8, 456 (2017).
31. Peters, B. A. et al. Relating the gut metagenome and metatranscriptome to immunotherapy responses in melanoma patients. Genome Med. 11, 61 (2019).
32. Matson, V. et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 359, 104–108 (2018).
33. Geva-Zatorsky, N. et al. Mining the human gut microbiota for immunomodulatory organisms. Cell 168, 928–943.e11 (2017).
34. Smith, M. et al. Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. Nat. Med. 28, 713–723 (2022).
35. Botticelli, A. et al. Changes of microbiome profile during nivolumab treatment in NSCLC patients. J. Clin. Oncol. 36, e15020-e15020 (2018).
36. Schirmer, M. et al. Linking the human gut microbiome to inflammatory cytokine production capacity. Cell 167, 1897 (2016).
37. Ashraf, R. & Shah, N. P. Immune system stimulation by probiotic microorganisms. Crit. Rev. Food Sci. 54, 938–956 (2014).
38. He, F. et al. Intestinal Bifidobacterium species induce varying cytokine production. J. Allergy Clin. Immunol. 109, 1035–1036 (2002).
39. Blacher, E., Levy, M., Tatirovsky, E. & Elinav, E. Microbiome-modulated metabolites at the interface of host immunity. J. Immunol. 198, 572–580 (2017).
40. Matare, G. et al. Procalcitonin neutralizes bacterial LPS and reduces LPS-induced cytokine release in human peripheral blood mononuclear cells. BMC Microbiol. 12, 68 (2012).
41. Wong, M. H., Chapin, O. C. & Johnson, M. D. LPS-stimulated cytokine production in type I cells is modulated by the renin-angiotensin system. Am. J. Resp. Cell Mol. Biol. 46, 641–650 (2012).
42. Dziarski, R. Recognition of bacterial peptidoglycan by the innate immune system. Cell Mol. Life Sci. 60, 1793–1804 (2003).
43. Li, P., Yin, Y. L., Li, D., Kim, S. W. & Wu, G. Y. Amino acids and immune function. Br. J. Nutr. 98, 237–252 (2007).
44. Sikalidis, A. K. Amino acids and immune response: a role for cysteine, glutamine, phenylalanine, tryptophan and arginine in T-cell function and cancer? Pathol. Oncol. Res. 21, 9–17 (2015).
45. Grohmann, U. & Bronte, V. Control of immune response by amino acid metabolism. Immunol. Rev. 236, 243–264 (2010).
46. Xiao, N. et al. Integrated cytokine and metabolite analysis reveals immunometabolic reprogramming in COVID-19 patients with therapeutic implications. Nat. Commun. 12, 1618 (2021).
47. Durie, B. G. et al. International uniform response criteria for multiple myeloma. Leukemia 20, 1467–1473 (2006).
48. Rajkumar, S. V. et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood 117, 4691–4695 (2011).
49. Hu, Y. X. et al. Potent anti-leukemia activities of chimeric antigen receptor-modified T cells against CD19 in Chinese patients with relapsed/refractory acute lymphoblastic leukemia. Clin. Cancer Res. 23, 3297–3306 (2017).
50. Wang, Y. et al. A retrospective comparison of CD19 single and CD19/CD22 bispecific targeted chimeric antigen receptor T cell therapy in patients with relapsed/refractory acute lymphoblastic leukemia. Blood Cancer J. 10, 105 (2020).
51. Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahe, F. ViSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584 (2016).
52. Bolyen, E. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857 (2019).
53. Amir, A. et al. Deblur rapidly resolves single-nucleotide community sequence patterns. Msysystems 2, e00191-16 (2017).
54. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2—a approximately maximum-likelihood trees for large alignments. PLoS ONE 5, e9490 (2010).
55. Bokulich, N. A. et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin. Microbiome 6, 10 (2018).
56. Douglas, G. M. et al. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688 (2020).
57. Bokulich, N. A. et al. q2-longitudinal: longitudinal and paired-sample analyses of microbiome data. mSystems 3, e00219-18 (2018).
58. Nueda, M. J., Tarazona, S. & Conesa, A. Next maSigPro: updating maSigPro bioconductor package for RNA-seq time series. Bioinformatics 30, 2598–2602 (2014).
59. Conesa, A., Nueda, M. J., Ferrer, A. & Talon, M. maSigPro: a method to identify significantly differential expression profiles in time-course microarray experiments. Bioinformatics 22, 1096–1102 (2006).
60. Shannon, P. et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504 (2003).

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Author contributions
H.H. designed and supervised the clinical study; M.D.L. designed and supervised data analysis; M.M. designed the clinical study; H.C., Y.Z., and Mrs. Judith Gunn Bronson of Stem Line Publishing Services, Inc. for their editing of this manuscript.

Competing interests
The authors declare no competing interests.
