Immunoglobulin Isotype Switch after Anti-BCMA CAR T-Cell Therapy for Relapsed or Refractory Multiple Myeloma

Tracking no: ADV-2021-005814R1

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Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: ZL, PL, LK, WQ and AL were responsible for study design, data interpretation, and data analysis. ZL and PL wrote the manuscript. All authors recruited patients and contributed to data collection. All authors reviewed, edited, and approved the manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: The datasets generated/analyzed during the current study are available. Please contact to the corresponding author by email.

Clinical trial registration information (if any): This study was registered with ClinicalTrials.gov (NCT 04500431)
Title: Immunoglobulin Isotype Switch after Anti-BCMA CAR-T Cell Therapy for Relapsed or Refractory Multiple Myeloma

Running title: Immunoglobulin isotype switch and anti-BCMA CAR-T cell therapy

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To the Editor:

The malignant plasma cell clone in patients with multiple myeloma (MM) is represented by the production of paraprotein, usually a single abnormal unique monoclonal heavy and/or light chain with constant isotype. Switching the paraprotein or transient presence of oligoclonal bands (OB) on serum immunofixation electrophoresis (IFE) occurs in up to 73% patients after autologous stem cell transplantation (ASCT) and high-dose chemotherapy. The emergence of these abnormal protein band (APB) may represent a more durable immune reconstitution and be associated with a favorable prognosis in that setting.

B cell maturation antigen (BCMA)-specific chimeric antigen receptor T (CAR-T) cell therapy is emerging as a promising treatment for patients with relapsed or refractory (r/r) MM. The BCMA CAR-T cell therapy results in high overall response rates (ORR) (range, 64%-88%) in recent multiple clinical trials. CAR-T cell therapy, in common with ASCT and high-dose chemotherapy, involves the activation and regulation of the immune system. Specifically, plasma cells and mature B lymphocytes targeted by BCMA CAR-T cells are also integral components of the humoral immune system. However, the prevalence and clinical significance of APB in MM patients after CAR-T cell therapy remains unclear.

Between Jan 1st 2018 and Feb 1st 2020, 12 consecutive patients with r/r MM had been enrolled into a clinical trial of BCMA CAR-T cells (NCT04500431). This study was approved by the Tongji Hospital of Tongji University Ethics Committee and written informed consent was provided by each patient. All studies were conducted in accordance with the Declaration of Helsinki. Full details on BCMA CAR-T manufacturing and clinical procedure are in the Supplemental Methods. Of the 12 patients treated, the overall response (OR) rate was 75% (9/12) with strict complete response (sCR) 67% (8/12) and very good partial response (VGPR) 8% (1/12) (Figure 1A). Quantitative real-time polymerase chain reaction (qPCR) showed those patients with response (partial response or better) had a peak in CAR transgene copies in
peripheral blood between days 7-21, and then dropped to low or undetectable levels by 6 months (Figure S1). With a median follow-up of 15 months (range, 4-26), the median progression-free survival (PFS) and the median overall survival (OS) were 12 months and not reached, respectively (Figure 1B and 1C). The adverse events within 30 days after infusion were well tolerated. Hematologic toxic effects were the most common events of grade 3 or higher, including neutropenia (67%), anemia (42%), thrombocytopenia (33%). A total of 11 patients (92%) had cytokine release syndrome (CRS), which was of grade 1 or 2 in 9 patients (75%) and grade 3 in 2 patients (17%). Only 1 patient experienced grade 1 neurologic toxicity. Three patients (25%) received tocilizumab and (or) glucocorticoids. No patients experienced severe infection within 30 days after infusion. Detailed baseline characteristics, treatment response, and toxicities of these patients are described in Table S1 and S2.

During the follow-up after CAR-T cell therapy, we observed that 4 (33%) patients had APB on serum IFE distinct from the paraprotein present at diagnosis, which were below the level of detection (0.1g/dL) for all measurements by serum protein electrophoresis (Table 1 and Figure S2). The baseline characteristics such as age, extramedullary disease, prior lines of therapy and tumor burden in the 4 patients with APB were similar to those in patients without APB (Table S3). Several studies reported that APB emerged during immunomodulatory and (or) bortezomib-combined therapies as well as after ASCT.\textsuperscript{13,14} In this study, none of these patients with APB received ASCT prior to CAR-T cell therapy, and the intervals from last treatment to CAR-T infusion in the 4 patients were longer than 3 months (Table 1), suggesting that those agents are less likely sole contributors to APB occurrence. Of the 4 patients with APB, 2 patients had only isotype switch (IS), and 2 had both IS and OB. All IS occurred with clones that made IgG\textsubscript{κ} and (or) IgG\textsubscript{λ} (Figure S2A-D). The median time to the occurrence of APB from infusion was 4 months after CAR-T infusion (range, 2 to 6 months). The median duration of APB was 5 months (range, 3 to 9 months).
At the detection of APB, all the 4 patients were completely asymptomatic and free of CRAB (hypercalcemia, renal failure, anemia, and bone disease) complications. Bone marrow (BM) examination showed that BM morphology was normal and there was no evidence of a neoplastic plasma cell population by flow cytometric analysis at the time of APB. Patient TJ12 had 2 abdominal masses before CAR-T cell infusion that resolved on positron emission tomography–computed tomography (PET-CT) during APB occurred (Figure S2E). The above results suggested the APB was not associated with relapse of disease or emergence of a clone of malignant plasma cells.

APB occurrence has been shown to correlate with superior survival among patients with MM who have undergone ASCT.\textsuperscript{5,6} In this study, we performed a post-hoc analysis of the clinical outcome between patients with APB and patients without APB to determine if APB also had prognostic significance in post-CAR-T setting. Interestingly, all the 4 patients with APB achieved sCR (100%) within 6 months, and the remission states were sustained at the last follow-up. Of those without APB, 5 patients achieved an objective response with 4 sCR (50%) and 1 VGPR (12.5%) (Figure 1D). With a median follow-up of 15 months, the median OS has not been reached in patients with and without APB (p=0.56), however, the median PFS in patients without APB was only 7 months while the median PFS in patients with APB was not reached (P=0.04) (Figure 1B and 1C).

The etiology of the APB phenomenon remains unclear. The appearance of APB could be associated with immune reconstitution and recovery of B cell function, as a result of the alleviation of the myeloma-induced immunosuppressive effects after high-dose chemotherapy.\textsuperscript{4,7} We hypothesized that similar mechanism could underlie the phenomenon of APB occurring after BCMA CAR-T therapy. Therefore, we analyzed the changes of blood immune cell subsets pre- and post-CAR-T cell therapy in the cohort of patients with APB. At baseline, the number of circulating CD19+ B cells in those patients were below normal levels, which is a very common condition in MM patients with disease progression. At time point greater than 3 months after
CAR-T cell infusion, patients had normal and greater than normal numbers of circulating B cells (Figure 1E). Although the absolute number of blood T cells did not change significantly after CAR-T cell therapy (Figure 1F), the CD4/CD8 ratios were inverted from 1.54 at baseline to 0.58 at 3 months and 0.81 at 6 months (Figure 1H), which was related to an increase in CD4+ T cell percentage and a decrease in CD8+ T cell percentage (Figure 1I and 1J). There are no significant changes in the absolute numbers of NK cells before and after CAR-T cell therapy (Figure 1G). These above results suggested that the APB occurrence after CAR T-cell therapy could be a transient process accompanied by restoration of B cell function and T cell immune reconstitution. Due to the limited data in this study, the subpopulation of B cell responsible for production of the paraprotein could not be defined, and further studies are needed to elucidate the precise source of the paraprotein.

In summary, this is the first report of APB phenomenon in patients with r/r MM after BCMA CAR T-cell therapy. Our results showed the incidence of APB is 33.3%, and the patients with APB had higher percentage of CR and a better outcome compared to those without APB. Our results also suggest that APB is more likely a temporary benign phenomenon associated with restoration of B cell function and immune reconstitution rather than malignant transformation. Thus, it is important for practitioners to clarify the clinical significance of APB occurrence after BCMA CAR-T cell therapy and obviates the inappropriate consideration of salvage therapy. The limitation of this study is a small sample size. The true incidence and clinical significance of APB occurrence after CAR-T cell therapy warrant further validation with larger number of patients in prospective clinical trials.
Acknowledgments

This work was supported by funds from the National Natural Science Foundation of China (Nos. 81830004, 81830006 and 82070168), Translational Research Grant of NCRCH (2020ZKZC04) and Science Foundation of Shanghai Municipal Health Commission (2020CXJQ02).

Authorship

Contribution: ZL, PL, LK, WQ and AL were responsible for study design, data interpretation, and data analysis. ZL and PL wrote the manuscript. All authors recruited patients and contributed to data collection. All authors reviewed, edited, and approved the manuscript.

Conflict-of-interest disclosure: All authors declare that they have no conflict of interest.

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Data Availability Statement

The datasets generated/analyzed during the current study are available. Please contact to the corresponding author by email: lab7182@tongji.edu.cn.
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### Table 1. The isotype changes and characteristics in the 4 patients with APB

| ID  | Sex | Age | Primary monoclonal protein | ASCT | Last-treatment line regimen | Time from last-line treatment to CAR-T infusion (months) | Clonal band pattern | Time from infusion to onset of APB (months) | APB duration (months) | Clinical response | PFS (months) |
|-----|-----|-----|-----------------------------|------|------------------------------|----------------------------------------------------------|---------------------|---------------------------------------------|-----------------------|-----------------|--------------|
| TJ02 | M   | 73  | IgG λ                        | No   | TD                          | 3                                                        | IgG λ → IgG κ+ IgG λ → IgG κ → normal                   | 3                                           | 9                            | sCR           | 25           |
| TJ09 | F   | 51  | IgD λ                        | No   | RCD                         | 6                                                        | (IgD) λ → IgG λ → IgG κ+ IgG λ → normal                  | 2                                           | 7                            | sCR           | 12           |
| TJ10 | M   | 49  | IgG λ                        | No   | VDEP                        | 3                                                        | IgG λ → Oligo IgG κ+ IgG λ → normal                     | 6                                           | 3                            | sCR           | 9            |
| TJ12 | M   | 53  | κ light chain                | No   | VRd                         | 3                                                        | κ → normal → IgG κ+ Oligo IgG λ → normal              | 5                                           | 3                            | sCR           | 8            |

ASCT: autologous stem cell transplantation; APB, abnormal protein band; PFS, progression-free survival; F, female; M, male; sCR, strict complete response; TD, Thalidomide and Dexamethasone; RCD, Lenalidomide, Cyclophosphamide and Dexamethasone; VDEP, Bortezomib, Dexamethasone, Etoposide and Cisplatin; VRD, Bortezomib, Lenalidomide and Dexamethasone.

**Revised: Figure 1. Clinical response in patients with R/R MM to BCMA CAR-T cell therapy and immune signatures of patients with APB.** (A) Duration of response to CAR-T and postinfusion survival in 12 patients with r/r MM. Cyan dots indicate that patients developed APB after CAR-T infusion. (B-C) The PFS and OS of MM patients with or without APB after CAR-T therapy (mean±SEM). (D) The response of MM patients with or without APB after CAR-T therapy. (E-G) The count of blood B cells, T cells and NK cells (mean±SEM). (H) The ratio of CD4+ and CD8+ cells. (I-J) The percentage of CD4+ and CD8+ cells in blood lymphocytes. #Patients without APB vs. Patients with APB. * P < 0.05. PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response; CR, complete response; sCR, strict complete response; MRD, minimal residual disease; Pts, Patients; SEM, standard error of mean.
