Humoral immune reconstitution after anti-B-cell maturation antigen CAR-T cell therapy in patients with relapse/refractory multiple myeloma

Supplemental Data

Table S1. Classification of infection severity.

| Classification            | Definitions                                                                 |
|---------------------------|-----------------------------------------------------------------------------|
| Mild infections           | required no treatment                                                        |
| Moderate infections       | required oral treatment only                                                 |
| Severe infections         | required intravenous antimicrobial therapy or were associated with           |
|                           | other clinical circumstances that were considered severe, with the exception of |
|                           | bacteremia due to possible skin contaminants and fever                       |
|                           | without systemic symptoms (categorized as moderate)                          |
| Life-threatening infections| complicated by symptoms considered life-threatening                          |
| Fatal infections          | contributed significantly to death                                           |
Table S2. Univariate and multivariate Cox models for factors associated with B cell recovery after anti-BCMA CAR-T cell infusion.

| Factors                              | Univariate Hazard Ratio (95% CI) | P     | Multivariate Hazard Ratio (95% CI) | P     |
|--------------------------------------|----------------------------------|-------|------------------------------------|-------|
| Age (<60 years)                      | 1.79 (0.71-4.53)                 | 0.217 |                                   |       |
| Male gender                          | 0.80 (0.36-1.80)                 | 0.596 |                                   |       |
| ISS Stage                             |                                  |       |                                     |       |
| I versus II-III                      | 1.84 (0.8-4.24)                  | 0.151 | 2.53 (1.04-6.15)                   | 0.042 |
| Previous therapy lines (<4 lines)    | 2.18 (0.99-4.83)                 | 0.054 | 1.97 (0.87-4.47)                   | 0.104 |
| Costimulatory molecule               |                                  |       |                                     |       |
| CD28 versus 4-1BB                    | 0.42 (0.12-1.42)                 | 0.163 |                                   |       |
| CAR-T cell dose                      |                                  |       |                                     |       |
| (1-2) ×10^6 versus (1-2) ×10^7       | 0.64 (0.29-1.42)                 | 0.272 |                                   |       |
| CRS grade                            |                                  |       |                                     |       |
| 1-2 versus 3-5                       | 0.90 (0.33-2.41)                 | 0.831 |                                   |       |
| Tocilizumab use                      | 2.32 (1.03-5.24)                 | 0.043 | 2.53 (1.06-6.06)                   | 0.037 |
| Corticosteroids use                  | 1 (0.39-2.53)                    | 0.997 |                                   |       |

CI, confidence interval; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ISS, International Staging System.
Table S3. Factors associated with B cell recovery at 3-month* after anti-BCMA CAR-T cell infusion.

| Factors                                | Recovered at 3-mo (n=13) | Unrecovered at 3-mo (n=24) | Univariate P  | Multivariate P |
|----------------------------------------|---------------------------|-----------------------------|---------------|----------------|
| Age, years, median (range)             | 59 (40-70)                | 54 (27-70)                  | 0.175         | -              |
| Male gender, n (%)                     | 9 (69.23)                 | 15 (62.5)                   | 0.734         | -              |
| ISS Stage, n (%)                       |                           |                             |               |                |
| I                                      | 5 (38.46)                 | 9 (37.5)                    |               |                |
| II                                     | 5 (38.46)                 | 6 (25)                      |               |                |
| III                                    | 3 (23.08)                 | 9 (37.5)                    |               |                |
| Previous therapy lines, median (range)| 3 (2-8)                   | 5 (3-9)                     | 0.043         | 0.039          |
| Costimulatory molecule                 |                           |                             | 0.007         | 0.998          |
| CD28                                   | 0                         | 10 (41.67)                  |               |                |
| 4-1BB                                  | 13 (100)                  | 14 (58.33)                  |               |                |
| CAR-T cell dose, n (%)                 |                           |                             | 0.188         | -              |
| (1-2) ×10⁶ cells/kg                    | 5 (38.46)                 | 15 (62.5)                   |               |                |
| (1-2) ×10⁷ cells/kg                    | 8 (61.54)                 | 9 (37.5)                    |               |                |
| CRS grade, n (%)                       |                           |                             | 0.999         | -              |
| 1-2                                    | 11 (84.61)                | 19 (79.27)                  |               |                |
| 3-5                                    | 2 (15.38)                 | 4 (16.67)                   |               |                |
| Tocilizumab, n (%)                     | 6 (46.15)                 | 5 (3-9)                     | 0.143         | 0.12           |
| Corticosteroids, n (%)                 | 3 (23.08)                 | 8 (33.33)                   | 0.711         | -              |
| Median duration of persistent undetectable BCMA expression in BM (days) | 198                        | 246                         | 0.387         | -              |

BCMA, B-cell maturation antigen; BM, Bone marrow; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ISS, International Staging System; mo, month.

* 3 patients had unavailable CD19 detection at 3-month.
Table S4. Relationship between CAR-T cell persistence and recovery of immune parameters after anti-BCMA CAR-T cell infusion.

| Median recovery time of                | Duration of CAR-T cell persistence ≥ 6 mo (n=17) | Duration of CAR-T cell persistence < 6 mo (n=14) | P value |
|--------------------------------------|-----------------------------------------------|-----------------------------------------------|---------|
| B cell counts                        | 186                                           | 189                                           | 0.981   |
| Plasma cells                         | 368                                           | 142                                           | 0.0003  |
| Serum IgG concentrations             | 386                                           | 431                                           | 0.182   |
| Serum IgG concentrations             | 262                                           | 270                                           | 0.654   |
| Serum IgG concentrations             | Not reached                                   | 360                                           | 0.0009  |

BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; mo, month.
Table S5. Factors related to undetectable IgM in patients with non-IgM MM after anti-BCMA CAR-T cell infusion.

| Factors                              | Undetectable IgM n=21 | Detectable IgM n=19 | P      |
|--------------------------------------|-----------------------|---------------------|--------|
| Age, years, median (range)           | 53 (27-70)            | 56 (34-65)          | 0.723  |
| Male, n (%)                          | 14 (66.67)            | 13 (68.42)          | 0.999  |
| ISS stage, n (%)                     |                       |                     | 0.719  |
| I                                    | 7 (33.33)             | 8 (42.11)           |        |
| II                                   | 8 (38.10)             | 5 (26.32)           |        |
| III                                  | 6 (28.57)             | 6 (31.58)           |        |
| Previous therapy lines (median, range) | 4 (2-9)               | 4 (2-9)             | 0.406  |
| Costimulatory molecule               | 0.038                 |                     |        |
| CD28                                 | 3 (14.29)             | 9 (47.37)           |        |
| 4-1BB                                | 18 (85.71)            | 10 (52.63)          |        |
| CAR-T dose, n (%)                    |                       |                     | 0.545  |
| (1-2) ×10⁶/kg                        | 10 (47.62)            | 11 (57.89)          |        |
| (1-2) ×10⁷/kg                        | 11 (52.38)            | 8 (42.11)           |        |
| CRS grade, n (%)                     |                       |                     | 0.999  |
| 1-2                                  | 17 (80.95)            | 16 (84.21)          |        |
| 3-5                                  | 3 (14.29)             | 3 (15.79)           |        |
| Tocilizumab, n (%)                   | 7 (33.33)             | 4 (21.05)           | 0.488  |
| Corticosteroids, n (%)               | 4 (19.05)             | 8 (42.11)           | 0.170  |
| Median days until BCMA expression re-detected in BM | 225                   | 226                 | 0.887  |

BCMA, B-cell maturation antigen; BM, Bone marrow; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ISS, International Staging System.
Table S6. Factors related to undetectable IgA in patients with non-IgA MM after anti-BCMA CAR-T cell infusion.

| Factors                                    | Undetectable IgA n=23 | Detectable IgA N=6 | P    |
|--------------------------------------------|-----------------------|--------------------|------|
| Age, years, median (range)                 | 53 (27-70)            | 59 (49-75)         | 0.160|
| Male, n (%)                                | 15 (65.22)            | 6 (100)            | 0.148|
| ISS stage, n (%)                           |                       |                    | 0.646|
| I                                          | 8 (34.78)             | 3 (50)             |      |
| II-III                                     | 15 (65.22)            | 3 (50)             |      |
| Previous therapy lines (median, range)     | 5 (2-9)               | 3.5 (2-8)          | 0.311|
| Costimulatory molecule                     |                       |                    | 0.999|
| CD28                                       | 7 (30.43)             | 2 (40)             |      |
| 4-1BB                                      | 16 (69.57)            | 4 (60)             |      |
| CAR-T dose, n (%)                          |                       |                    | 0.080|
| (1.2) ×10^6/kg                             | 14 (60.87)            | 1 (16.67)          |      |
| (1.2) ×10^7/kg                             | 9 (39.13)             | 5 (83.33)          |      |
| CRS grade, n (%)                           |                       |                    | 0.568|
| 1-2                                        | 19 (82.61)            | 5 (100)            |      |
| 3-5                                        | 4 (17.39)             | 0                  |      |
| Tocilizumab, n (%)                         |                       |                    | 0.999|
| Corticosteroids, n (%)                     |                       |                    | 0.999|
| Median days until BCMA expression re-      |                       |                    | 0.443|
| detected in BM)                            | 225                   | 254                |      |

BCMA, B-cell maturation antigen; BM, Bone marrow; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ISS, International Staging System.
Table S7. Univariate and multivariate Cox models for factors associated with serum IgG, IgM and IgA recovery after anti-BCMA CAR-T cell infusion.

| Factors                              | IgG (Non-IgG MM patients, n=23) |                         | IgM (Non-IgM MM patients, n=40) |                         | IgA (Non-IgA MM patients, n=29) |                         |
|--------------------------------------|----------------------------------|-------------------------|----------------------------------|-------------------------|----------------------------------|-------------------------|
|                                      | Univariate                      | Multivariate           | Univariate                      | Multivariate           | Univariate                      | Multivariate           |
|                                      | Hazard Ratio (95% CI)            | Hazard Ratio (95% CI)   | Hazard Ratio (95% CI)            | Hazard Ratio (95% CI)   | Hazard Ratio (95% CI)            | Hazard Ratio (95% CI)   |
|                                      | P                                | P                       | P                                | P                       | P                                | P                       |
| Age (<60 years)                      | 0.53 (1.56-1.91)                 | 0.342                   | 1.37 (0.57-3.28)                 | 0.480                   | 2.68 (0.31-23.52)                 | 0.074                   |
| Male gender                          | 1.21 (0.31-3.98)                 | 0.846                   | 0.55 (0.25-1.21)                 | 0.136                   | 0.36 (0.07-1.86)                 | 0.225                   |
| ISS Stage                            |                                  |                         |                                  |                         |                                  |                         |
| I versus II-III                      | 0.17 (0.01-1.45)                 | 0.104                   | 0.2 (0.02-1.80)                  | 0.150                   | 0.17 (0.56-5.85)                 | 0.666                   |
| Previous therapy lines (<4)          | 0.98 (0.27-3.62)                 | 0.980                   | 2.22 (0.99-4.98)                 | 0.054                   | 2.26 (1.00-5.13)                 | 0.051                   |
| Costimulatory molecule               |                                  |                         |                                  |                         |                                  |                         |
| CD28 vs 4-1BB                        | 2.69 (0.68-10.63)                | 0.157                   | 0.64 (0.24-1.71)                 | 0.373                   | 1.07 (0.20-5.91)                 | 0.935                   |
| CAR-T cell dose                      | (1-2)×10^6 vs (1-2)×10^7         | 0.43 (0.12-1.53)        | 0.190                            | 1.16 (0.53-2.53)        | 0.719                            | 0.22 (0.03-1.89)        |
| CRS grade                            |                                  |                         |                                  |                         |                                  |                         |
| 1-2 versus 3-5                       | 0.44 (0.10-1.87)                 | 0.261                   | 1.07 (0.38-2.98)                 | 0.891                   | 1.24 (0.14-11.09)                | 0.831                   |
| Tocilizumab use                      | 4.38 (1.03-18.52)                | 0.045                   | 3.58 (0.85-15.21)                | 0.083                   | 0.86 (0.1-7.51)                  | 0.891                   |
| Corticosteroids use                  | 1.25 (0.30-5.24)                 | 0.765                   | 1.52 (0.67-3.43)                 | 0.310                   | 0.84 (0.1-7.32)                  | 0.877                   |

BCMA, B-cell maturation antigen; BM, Bone marrow; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ISS, International Staging System.
Table S8. Factors associated with serum IgG, IgM and IgA concentrations at 1-year after anti-BCMA CAR-T cell infusion.

| Factors                        | IgG concentrations at 1 year | P  | IgM concentrations at 1 year | P  | IgA concentrations at 1 year | P  |
|--------------------------------|-----------------------------|----|-----------------------------|----|-------------------------------|----|
|                                | Recovered | Unrecovered | Recovered | Unrecovered | Recovered | Unrecovered | Recovered | Unrecovered | Recovered | Unrecovered | Recovered | Unrecovered | Recovered | Unrecovered |
| Total                          | 8         | 7             | 19       | 7             | 5          | 16           |          |             |          |             |          |             |          |             |
| Age, years (median, range)     | 64 (28-70) | 62 (41-70)   | 53 (27-70) | 51 (44-65)   | 65 (49-70) | 51 (27-70)     | 0.953    | 0.942       | 0.082    |             |          |             |          |             |
| Male, n (%)                    | 4 (50)    | 4 (57.14)    | 12 (63.16) | 4 (57.14)    | 4 (80)     | 10 (62.5)    | 0.281    | 0.999       | 0.999    |             |          |             |          |             |
| ISS stage, n (%)               | 0.112     | 0.640        |          |              | 0.187      |              |          |             |          |             |          |             |          |             |
| I                              | 1 (12.5)  | 4 (57.14)    | 9 (47.37)  | 2 (28.57)    | 4 (80)     | 6 (37.5)     | 0.467    | 0.999       | 0.999    |             |          |             |          |             |
| II                             | 1 (12.5)  | 1 (14.29)    | 5 (26.32)  | 3 (42.86)    | 1 (20)     | 6 (37.5)     |          |             |          |             |          |             |          |             |
| III                            | 6 (75)    | 2 (28.57)    | 5 (26.32)  | 2 (28.57)    | 0          | 4 (25)       |          |             |          |             |          |             |          |             |
| Previous therapy lines (median, range) | 3.5 (3-6) | 4 (2-8)       | 3 (2-9) | 6 (3-8) | 0.311 | 3 (2-3) | 5 (2-9) | 0.005 |
| Costimulatory molecule         |          |              | 0.467    |              | 0.999     |              |          |             |          |             |          |             |          |             |
| CD28                           | 2 (25)    | 0             | 5 (26.32)  | 2 (28.57)    | 1 (20)     | 5 (31.25)    |          |             |          |             |          |             |          |             |
| 4-1BB                          | 6 (75)    | 7 (100)       | 14 (73.68) | 5 (71.43)    | 4 (80)     | 11 (68.75)   |          |             |          |             |          |             |          |             |
| CAR-T dose, n (%)              | 0.282     |              | 0.999     |              |          |             | 0.311    |              |          |             |          |             |          |             |
| (1-2) × 10^6/kg                | 4 (50)    | 6 (85.71)     | 9 (47.37)  | 4 (57.14)    | 1 (20)     | 9 (56.25)    |          |             |          |             |          |             |          |             |
| (1-2) × 10^7/kg                | 4 (50)    | 1 (14.29)     | 10 (52.63) | 3 (42.86)    | 4 (80)     | 7 (43.75)    |          |             |          |             |          |             |          |             |
| CRS grade, n (%)               | 0.580     |              | 0.588     |              |          |             | 0.999    |              |          |             |          |             |          |             |
| 1-2                            | 5 (62.65) | 5 (71.43)     | 16 (84.21) | 5 (71.43)    | 4 (80)     | 14 (87.5)    |          |             |          |             |          |             |          |             |
| 3-5                            | 3 (37.57) | 1 (14.29)     | 3 (15.79)  | 2 (28.57)    | 1 (10)     | 2 (12.5)     |          |             |          |             |          |             |          |             |
| Tocilizumab, n (%)             | 2 (25)    | 2 (28.57)     | 6 (31.58)  | 1 (16.67)    | 0.629      | 1 (20)       | 0.999    |              |          |             |          |             |          |             |
| Corticosteroids, n (%)         | 4 (50)    | 1 (14.29)     | 5 (26.32)  | 1 (16.67)    | 0.647      | 1 (20)       | 0.999    |              |          |             |          |             |          |             |
| Median days until BCMA expression re-detected in BM | 200   | 208          | 0.967   | 210          | 0.689    | 121          | 0.029    |              |          |             |          |             |          |             |

BCMA, B-cell maturation antigen; BM, Bone marrow; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ISS, International Staging System.

* In patients with non- IgG MM; † In patients with non- IgM MM; ^ In patients with non- IgA MM. & ISS stage I compared with stage II and III.
| Characteristic                                      | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 |
|----------------------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Age, y                                              | 65        | 61        | 62        | 65        | 65        | 70        |
| Gender                                             | Female    | Male      | Male      | Male      | Female    | Male      |
| Subtype (monoclonal globulin)                      | IgG       | IgA       | IgA       | IgD       | IgA       | Kappa     |
| ISS Stage                                          | I         | II        | 1         | 1         | III       | III       |
| Previous therapy lines                             | 3         | 6         | 3         | 2         | 5         | 3         |
| CAR-T cell dose, cells/kg                          | 1×10⁷     | 1×10⁶     | 1×10⁶     | 1×10⁶     | 1×10⁶     | 1×10⁶     |
| BCMA re-detectable time, mo                        | 6.3       | 4.1       | 8.1       | 4.7       | 5.1       | 7.1       |
| B cell recovery before CAR-T cell function loss    | Yes       | Yes       | Yes       | Yes       | No, unrecovered at last follow-up | Yes |
| Date of last evaluation after CAR-T cell function loss, mo | 35.8      | 13.1      | 11.5      | 9.7       | 6.5       | 6         |
| IgG returning to normal level                      | NA        | No        | No        | No        | Yes, 6 mo after CAR-T cell loss | Yes, 3.7 mo after CAR-T cell loss |
| IgM returning to normal level                      | Yes, at the time of BCMA re-detectable | Yes, at the time of BCMA re-detectable | Yes, at the time of BCMA re-detectable | Yes, 3 mo after CAR-T cell loss | Yes, 6 mo after CAR-T cell loss | Yes, at the time of BCMA re-detectable |
| IgA returning to normal level                      | Yes, 11.7 mo after CAR-T cell loss | NA        | NA        | No        | NA        | No        |

CAR, chimeric antigen receptor; CR, complete response; CRS, cytokine release syndrome; ISS, International Staging System; mo, month; NA, not applicable; PR, partial response; sCR, stringent complete response; VGPR, very good partial response.
Table S10. Infections after anti-BCMA CAR-T cell infusion.

| Event                  | No. of patient (%) | Event                  | No. of patient (%) | Event                  | No. of patient (%) | Event                  | No. of patient (%) |
|------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|
| Any infection          | 19                 | 7                      | 6                  | 12                     | 8                  |
| Infection Severity     |                    |                        |                    |                        |                    |
| Mild/Moderate          | 8                  | 4                      | 4                  | 3                      | 5                  |
| Severe/Life-threatening| 11                 | 3                      | 2                  | 2                      | 7                  |
| Infection microorganism|                    |                        |                    |                        |                    |
| Bacteria               | 12                 | 5                      | 3                  | 5                      | 5                  |
| Fungi                  | 1                  | 1                      | 0                  | 1                      | 1                  |
| Viruses                | 2                  | 1                      | 2                  | 3                      | 3                  |
| Mycoplasma             | 0                  | 0                      | 0                  | 1                      | 1                  |
| Unclear                | 3                  | 3                      | 1                  | 1                      | 1                  |
| Infection site         |                    |                        |                    |                        |                    |
| Lung                   | 10                 | 5                      | 3                  | 5                      | 5                  |
| Bloodstream            | 3                  | 0                      | 0                  | 1                      | 1                  |
| URI                    | 2                  | 1                      | 2                  | 4                      | 4                  |
| Others                 | 4                  | 1                      | 1                  | 2                      | 1                  |

URI, Upper respiratory infection.

*At least one patient had >1 infections. *One patient also had mild/moderate infections. ^One patient had 2 bacterial infections. †One patient had 1 bacterial infection, 1 mycoplasma infection and 2 unknown infection. §One patient had 1 lung infection, 1 URI and 2 gastroenteritis. & Two gastroenteritis, one gingivitis, and one urinary infection within 60 days, one skin infection during days 60-120, one phlebitis of lower extremities during days 120-180, two repeated gastroenteritis over days 180 after CAR-T cell infusion.
Figure S1. Kaplan-Meier curves of progression-free survival and overall survival. (A) Progression free survival and (B) Overall survival for patients with R/R MM after anti-BCMA CAR-T cell therapy by Kaplan-Meier curves.
Figure S2. Kinetics of immune parameters recovery. Kinetics of B cell count (A), serum IgG (B), IgM (C) and IgA (D) concentrations in individual patient at different time points before and after CAR-T cell infusion.
Figure S3. Correlation between B cell count and other immune parameters. Scatter plots of B cell count to absolute lymphocyte count (ALC) (A), CD4+ T (B), CD8+ T (C), and NK (D) cell count and IgG (E), IgA (F) and IgM (G) concentrations from the same sample. IgG concentrations were analyzed in patients with non-IgG MM, IgM concentrations were analyzed in patients with non-IgM MM and IgA concentrations were analyzed in patients with non-IgA MM. Each point represents data from a single patient. r values were determined using the Spearman correlation test.
Figure S4. **CAR-T cell kinetics in individual subject.** Kinetics of CAR+ T cells with the costimulatory domain of 4-1BB (A) and CD28 (B) in individual patient. Numbers of anti-BCMA CAR-T cells in peripheral blood (PB) were assessed by FACS at serial time points before and after infusion of CAR-T cells in individual patient.
Figure S5. Immune reconstitution in patients whose progression-free survival (PFS) were <1 year and whose PFS were ≥1 year. Box and whisker plots of B cell count (A), serum IgG (B), IgM (C) and IgA (D) concentrations in patients whose PFS were <1 year and patients whose PFS were ≥1 year by timepoints from start of CAR-T cell infusion. IgG concentrations were analyzed in patients with non- IgG MM, IgM concentrations were analyzed in patients with non- IgM MM and IgA concentrations were analyzed in patients with non- IgA MM. Grey area indicates normal range. *P-values are calculated by the Kruskal-Wallis test. *P <0.05, **P <0.01.
Figure S6. Progression-free survival of patients with recovered immunoglobulin or not after anti-BCMA CAR-T cell infusion. (A) Progression-free survival (PFS) of non-IgG MM patients with recovered IgG concentrations or not at 1-year; (B) PFS of non-IgM MM patients with recovered IgM concentrations or not at 1-year; (C) PFS of non-IgA MM patients with recovered IgA concentrations or not at 1-year after anti BCMA CAR-T cell infusion.