Supplementary data

Improved clinical outcome in a randomized phase 2 study of anti-PD-1 camrelizumab plus decitabine in relapsed/refractory Hodgkin lymphoma

Yang Liu, Chunmeng Wang, Xiang Li, Liang Dong, Qingming Yang, Meixia Chen, Fengxia Shi, Malcolm V. Brock, Miao Liu, Qian Mei, Jiejie Liu, Jing Nie, Weidong Han

Liu Y, *et al. J Immunother Cancer* 2021; 9:e002347. doi: 10.1136/jitc-2021-002347

Contents

Supplementary Methods 3

Flow cytometry analysis 3

Global DNA methylation detection 3

Supplementary Figures 4

Figure 1. Duration of response in patients with tumor burden SPD ≥ 30 cm^2. 4

Figure 2. Progression-free survival in patients with higher tumor burden. 5

Figure 3. Peripheral biological parameters in patients after camrelizumab monotherapy or decitabine-plus-camrelizumab therapy. 6

Figure 4. Association of baseline percentage of peripheral T-cell subsets with progression-free survival after decitabine-plus-camrelizumab. 8

Figure 5. Association of percentage alteration of peripheral T-cell subsets with progression-free survival after decitabine-plus-camrelizumab or camrelizumab monotherapy. 10

Supplementary Tables 12

Table 1. Complete remission rate within subgroups. 12

Table 2. Characterization of patients who remain in remission after camrelizumab monotherapy. 13

Table 3. Baseline frequency of circulating T-cell subsets between the two groups. 14

Table 4. Baseline frequency of circulating T-cell subsets with CR rate in patients after camrelizumab monotherapy or decitabine-plus-camrelizumab combination. 15

Table 5. Immune-related adverse events in the overall population of 61 patients. 16

Supplementary Methods

Flow cytometry analysis

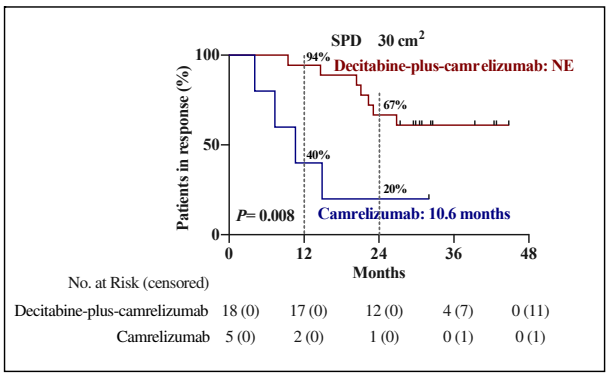
The peripheral blood was collected in sodium heparin anticoagulant vacutainer tubes. After red blood cell lysis and washing, cells were stained with the indicated antibodies according to the manufacture's instruments, and detected on a BD FACSCalibur flow cytometer (BD Biosciences), a minimum of 10,000 $CD3^+$ lymphocytes were collected. Following antibodies were purchased from BD Biosciences: anti-CD3-PerCP (347344), anti-CD8-APC (340584), anti-CCR7-PE (560765), anti-CD45RA-FITC (347723) and isotype-matched antibodies

Global DNA methylation detection

Blood samples from patients in both groups were collected at baseline (C1d0), before the second treatment-cycle (C2d0) and before the third treatment-cycle (C3d0). The peripheral blood mononuclear cells (PBMCs) were isolated. The global DNA methylation levels were measured by using the Global DNA Methylation LINE-1 kit (Active Motif, USA) according to the manufacture's instruction.

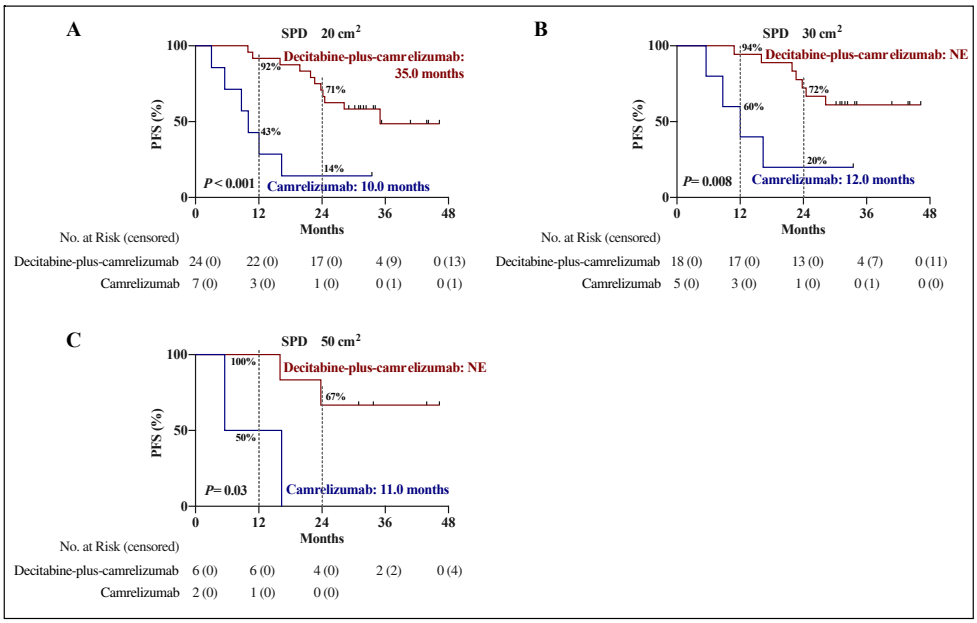
Supplementary Figures

Figure 1. Duration of response in patients with tumor burden SPD ≥ 30 cm^2.



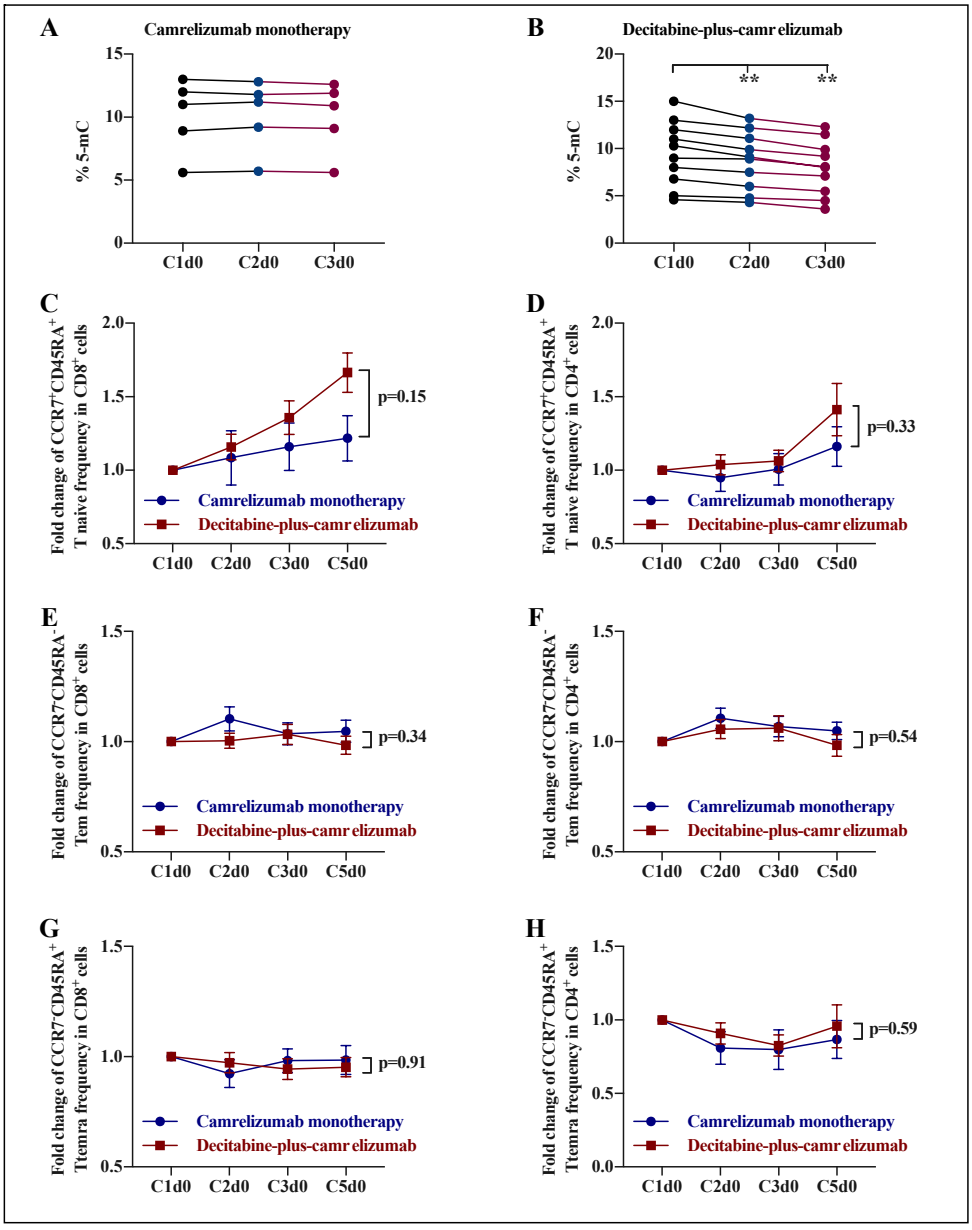
Kaplan-Meier estimates of response duration in responders who had tumor burden SPD ≥ 30 cm^2. The red curves represent patients treated with decitabine-plus-camrelizumab and the blue those treated with camrelizumab monotherapy. The median duration of response and duration of response rates at 12 months and 24 months were shown. Plus signs indicate censored. SPD, sum of the products of diameters.

Figure 2. Progression-free survival in patients with higher tumor burden.



Kaplan-Meier estimates of progression-free survival in patients who had tumor burden SPD ≥ 20 cm^2 (A), SPD ≥ 30 cm^2 (B), or SPD ≥ 50 cm^2 (C). The red curves represent patients treated with decitabine-plus-camrelizumab and the blue those treated with camrelizumab monotherapy. The median progression-free survival and progression-free survival rates at 12 months and 24 months were shown. Plus signs indicate censored. SPD, sum of the products of diameters.

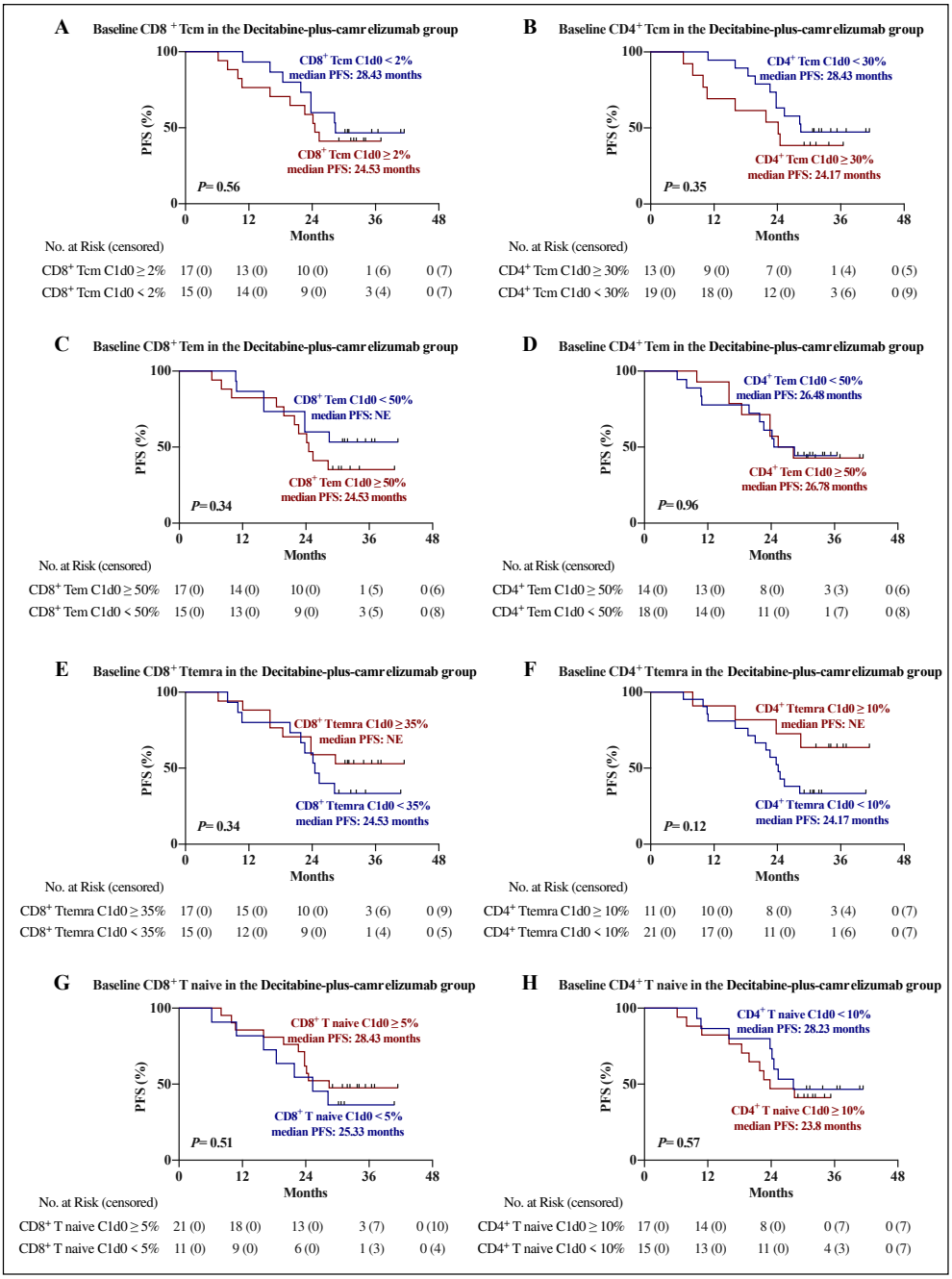
Figure 3. Peripheral biological parameters in patients after camrelizumab monotherapy or decitabine-plus-camrelizumab therapy.



(A, B) Global DNA methylation levels in PBMCs. PBMC samples at baseline (C1d0), before the second cycle (C2d0) and the third cycle (C3d0) from five patients in the camrelizumab group (A) and ten patients in the decitabine-plus-camrelizumab group (B) were collected, and the percentages of 5-mC were detected as compared with the total cytosine content at the indicated times, using the Global DNA Methylation LINE-1 kit. (C-D) Fold change of percentages of peripheral

$CCR7^+CD45RA^+$ T naïve cells in $CD8^+$ (or $CD4^+$) T-cells at the indicated times compared to baseline (C1d0) in the camrelizumab group or decitabine-plus-camrelizumab group, analyzed by FACS. (E-F) Fold change of percentages of peripheral $CCR7^-CD45RA^-$ Tem cells in $CD8^+$ (or $CD4^+$) T-cells at the indicated times compared to baseline (C1d0) in the camrelizumab group or decitabine-plus-camrelizumab group, analyzed by FACS. (G-H) Fold change of percentages of peripheral $CCR7^-CD45RA^+$ Ttemra cells in $CD8^+$ (or $CD4^+$) T-cells at the indicated times compared to baseline (C1d0) in the camrelizumab group or decitabine-plus-camrelizumab group, analyzed by FACS. The two-way repeated-measures analysis of variance (ANOVA) was conducted to evaluate the effect of time-group interaction, and p value was shown.

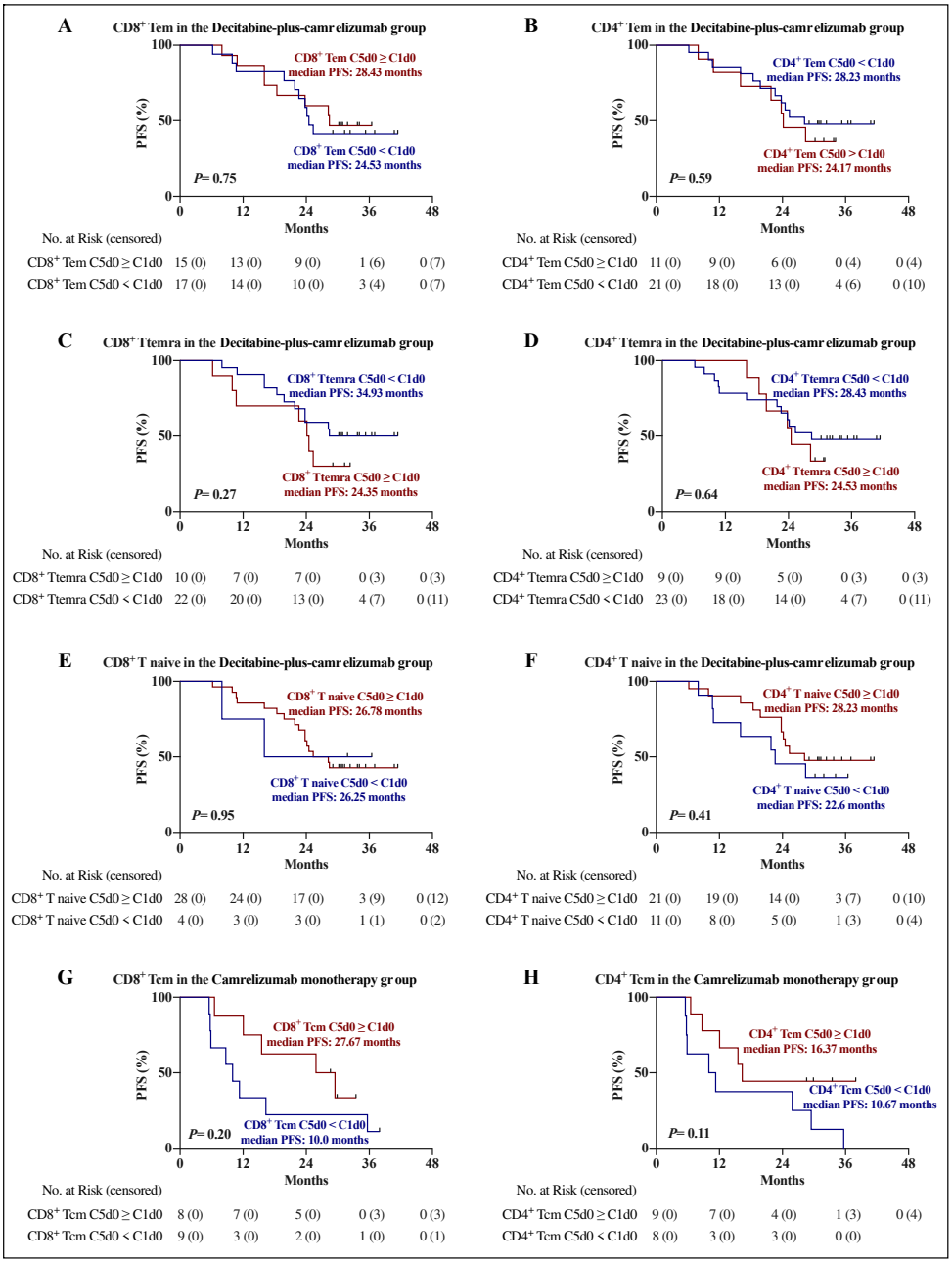
Figure 4. Association of baseline percentage of peripheral T-cell subsets with progression-free survival after decitabine-plus-camrelizumab.



Progression-free survival among subgroups according to the indicated baseline peripheral T-cell subsets ratios, $CCR7^+CD45RA^-CD8^+$ ($CD8^+$ Tcm [A]), $CCR7^+CD45RA^-CD4^+$ ($CD4^+$ Tcm [B]), $CCR7^-CD45RA^-CD8^+$ ($CD8^+$ Tem [C]), $CCR7^-CD45RA^-CD4^+$ ($CD4^+$ Tem [D]), $CCR7^-CD45RA^+CD8^+$

($CD8^+$ Ttemra [E]), $CCR7^-CD45RA^+CD4^+$ ($CD4^+$ Ttemra [F]), $CCR7^+CD45RA^+CD8^+$ ($CD8^+$ T naive [G]), $CCR7^+CD45RA^+CD4^+$ ($CD4^+$ T naive [H]). A *P* value < 0.05 was considered to indicate statistical significance.

Figure 5. Association of percentage alteration of peripheral T-cell subsets with progression-free survival after decitabine-plus-camrelizumab or camrelizumab monotherapy.



(A-F) Progression-free survival among subgroups according to the indicated peripheral T-cell subsets ratios detected on C5d0 as compared with C1d0 baseline levels in the decitabine-plus-camrelizumab group, $CCR7^-CD45RA^-CD8^+$ ($CD8^+$ Tem [A]), $CCR7^-CD45RA^-CD4^+$ ($CD4^+$ Tem [B]), $CCR7^-CD45RA^+CD8^+$ ($CD8^+$ Ttemra [C]), $CCR7^-CD45RA^+CD4^+$ ($CD4^+$ Ttemra [D]),

$CCR7^+CD45RA^+CD8^+$ ($CD8^+$ T naive [E]), $CCR7^+CD45RA^+CD4^+$ ($CD4^+$ T naive [F]). (G, H) Progression-free survival among subgroups according to $CCR7^+CD45RA^-CD8^+$ ($CD8^+$ Tcm [G]), $CCR7^+CD45RA^-CD4^+$ ($CD4^+$ Tcm [H]) ratios detected on C5d0 as compared with C1d0 baseline levels in the camrelizumab monotherapy group. A P value < 0.05 was considered to indicate statistical significance.

Supplementary Tables

Table 1. Complete remission rate within subgroups.

Subgroup	Camrelizumab monotherapy		Decitabine-plus-camrelizumab	
	No.	CR rate (95% CI) (%)	No.	CR rate (95% CI) (%)
Sex				
Male	12	25 (5-57)	25	84 (65-94)
Female	7	43 (10-82)	17	71 (47-87)
Age (years)				
< 28	8	38 (9-76)	21	76 (55-89)
≥ 28	11	27 (6-61)	21	81 (60-92)
Tumor histologic type				
NSHL	12	25 (5-57)	30	77 (59-88)
Non-NSHL	7	43 (10-82)	12	83 (55-95)
Tumor stage at initial diagnosis				
I/II	5	20 (1-72)	8	75 (41-93)
III/IV	14	36 (13-65)	34	79 (63-90)
Target lesions at enrollment				
Lymphoma nodes only	6	0 (0-46)	10	70 (40-89)
Extranodal involvement[a]	13	46 (19-75)	32	81 (65-91)
Tumor burden at enrollment[b]				
SPD < 20 cm^2	12	42 (15-72)	18	83 (61-94)
SPD ≥ 20 cm^2	7	14 (0-58)	24	75 (55-88)
Time from initial diagnosis				
< 2 years	7	29 (4-71)	24	71 (49-87)
≥ 2 years	12	33 (10-65)	18	89 (65-99)
Prior ASCT				
Yes	6	17 (0-64)	11	91 (59-100)
No	13	38 (14-68)	31	74 (55-88)
Previous lines of therapy				
< 3	4	50 (7-93)	12	92 (62-100)
≥ 3	15	27 (8-55)	30	73 (54-88)

[a] Sites of extranodal disease were bone, liver, spleen, lung, mediastinal mass and pleura.

[b] SPD, sum of the products of diameters.

Table 2. Characterization of patients who remain in remission after camrelizumab monotherapy.

Characteristics	In remission[a] (n=5)	Other responders[b] (n=12)
Median age in years (range)	30 (19-42)	29 (18-44)
Male	2 (40%)	9 (75%)
Disease stage at initial diagnosis		
Stage II	1 (20%)	4 (33%)
Stage III	1 (20%)	1 (8%)
Stage IV	3 (60%)	7 (58%)
Extranodal disease at enrollment	5 (100%)	7 (58%)
Primary refractory disease[c]	3 (60%)	4 (33%)
Tumor burden (SPD) at enrollment		
Median (range) (cm^2)	11 (6-31)	20 (3-96)
≥ 20 cm^2	1 (20%)	6 (50%)
Number of previous systemic therapy		
Median	3	3.5
≥ 3	3 (60%)	11 (92%)
Cycles of previous chemotherapy		
Median (range)	12 (8-30)	11 (6-21)
≥ 10	3 (60%)	8 (67%)
Previous autologous stem cell transplantation	0 (0)	5 (42%)
Previous brentuximab vedotin therapy	0 (0)	1 (8%)

Data are n (%), unless specified otherwise. SPD, sum of the products of diameters.

[a] Patients who achieve a CR and are still in remission without additional therapy.

[b] Patients with a best response of CR or PR who had subsequent progressive disease, or discontinued treatment since adverse events, or withdrawal of consent.

[c] Absence of CR or relapse within 90 days of the front-line therapy.

Table 3. Baseline frequency of circulating T-cell subsets between the two groups.

T-subset frequencies	Camrelizumab monotherapy (N=17)	Decitabine-plus-camrelizumab (N=32)	*P*-value
Baseline $CD8^+$ T_{cm}			
< 2%	8	15	1.00
≥ 2%	9	17	
Baseline $CD4^+$ T_{cm}			
< 30%	10	19	1.00
≥ 30%	7	13	
Baseline $CD8^+$ T_{em}			
< 50%	11	15	0.234
≥ 50%	6	17	
Baseline $CD4^+$ T_{em}			
< 50%	9	18	0.825
≥ 50%	8	14	
Baseline $CD8^+$ T_{naive}			
< 5%	8	11	0.386
≥ 5%	9	21	
Baseline $CD4^+$ T_{naive}			
< 10%	9	15	0.686
≥ 10%	8	17	
Baseline $CD8^+$ T_{temra}			
< 35%	7	15	0.703
≥ 35%	10	17	
Baseline $CD4^+$ T_{temra}			
< 10%	10	21	0.636
≥ 10%	7	11	

A P value < 0.05 was considered to indicate statistical significance. The P values were calculated in SPSS 18.0 using chi-square test.

Table 4. Baseline frequency of circulating T-cell subsets with CR rate in patients after camrelizumab monotherapy or decitabine-plus-camrelizumab combination.

T-subset frequencies	Camrelizumab Monotherapy (N=17)			Decitabine-plus-Camrelizumab (N=32)		
	CR	Non-CR	*P*-value	CR	Non-CR	*P*-value
Baseline $CD8^+$ T_{cm}						
< 2%	3	5	0.62	12	3	0.69
≥ 2%	2	7		12	5	
Baseline $CD4^+$ T_{cm}						
< 30%	3	7	1.00	17	2	**0.038**
≥ 30%	2	5		7	6	
Baseline $CD8^+$ T_{em}						
< 50%	3	8	1.00	7	3	0.68
≥ 50%	2	4		17	5	
Baseline $CD4^+$ T_{em}						
< 50%	4	5	0.29	13	5	1.00
≥ 50%	1	7		11	3	
Baseline $CD8^+$ T_{naive}						
< 5%	2	6	1.00	7	4	0.40
≥ 5%	3	6		17	4	
Baseline $CD4^+$ T_{naive}						
< 10%	2	7	0.62	12	3	0.69
≥ 10%	3	5		12	5	
Baseline $CD8^+$ T_{temra}						
< 35%	2	5	1.00	12	3	0.69
≥ 35%	3	7		12	5	
Baseline $CD4^+$ T_{temra}						
< 10%	2	8	0.59	15	6	0.68
≥ 10%	3	4		9	2	

A P value < 0.05 was considered to indicate statistical significance. The P values were calculated in SPSS 18.0 using chi-square test.

Table 5. Immune-related adverse events in the overall population of 61 patients.

Adverse Event	Decitabine-plus-camrelizumab (n=42)		Camrelizumab (n=19)	
	Any Grade	Grade 3-4	Any Grade	Grade 3-4
Any adverse event	15 (36)	0	5 (26)	1 (5)
Rash	5 (12)	0	0	0
Diarrhea	4 (10)	0	0	0
Myalgia	4 (10)	0	0	0
Hypothyroidism	3 (7)	0	3 (16)	0
Pneumonitis	3 (7)	0	1 (5)	1 (5)
hypoparathyroidism	1 (2)	0	0	0
Hypersensitivity	0	0	1 (5)	0