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Supplemental Information

Parallel Comparison of 4-1BB or CD28 Co-stimulated CD19-Targeted CAR-T Cells for B Cell Non-Hodgkin's Lymphoma

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Supplementary Information

The nucleotide sequence of 28z CAR

GACATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCA
GTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATTGGTATCAGCAGAAACCAGATGGAAC
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The nucleotide sequence of BBz CAR

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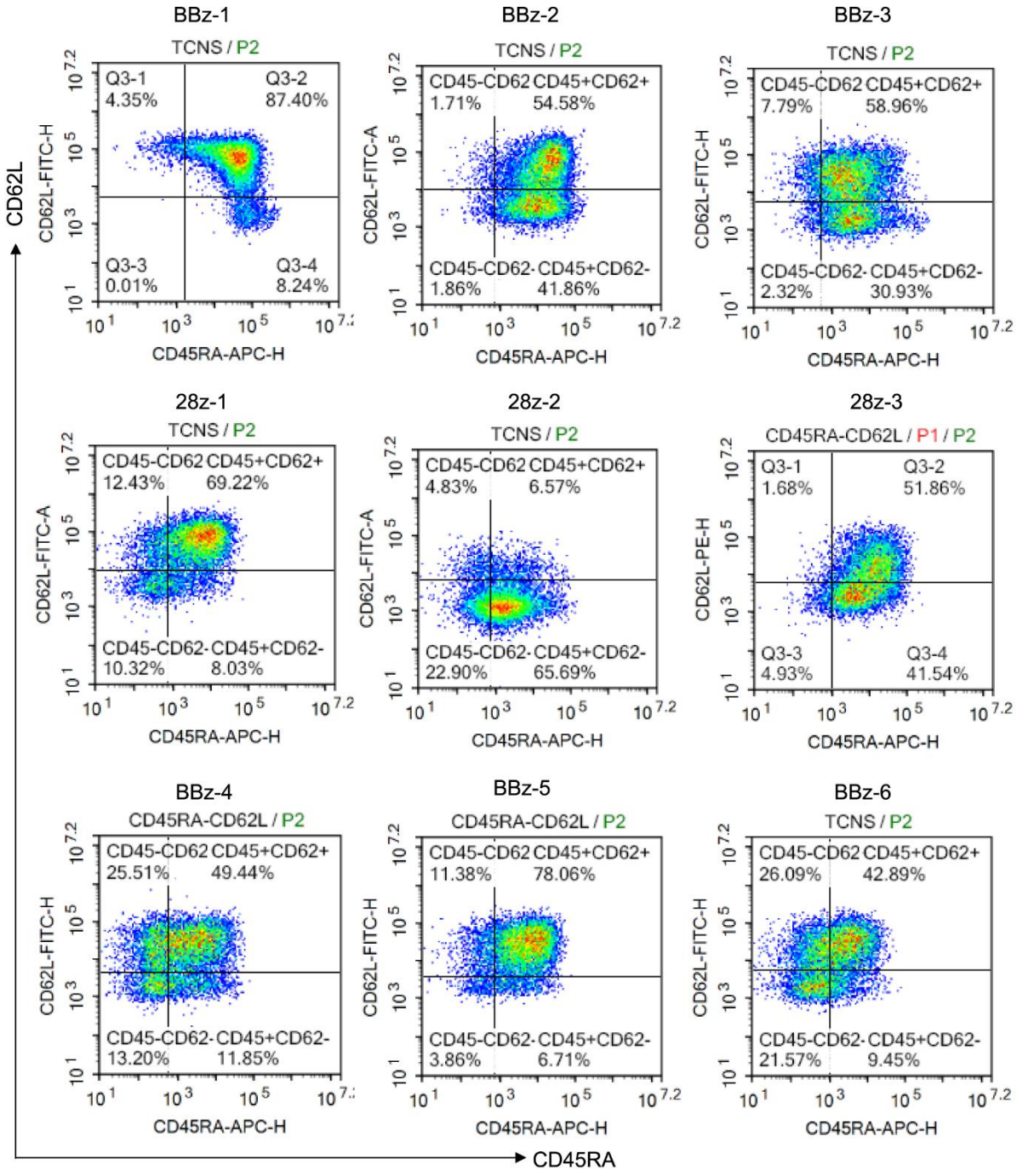
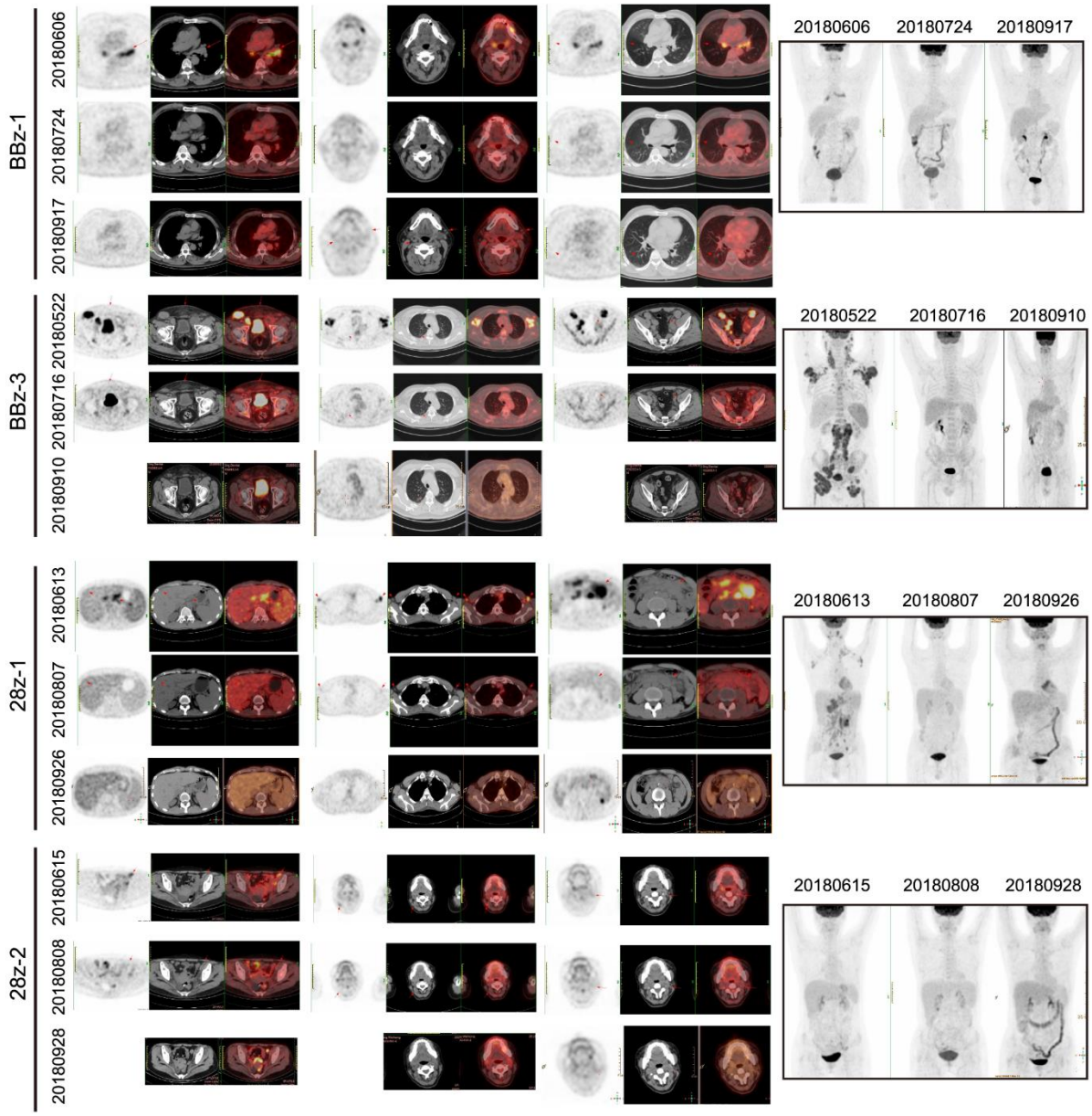
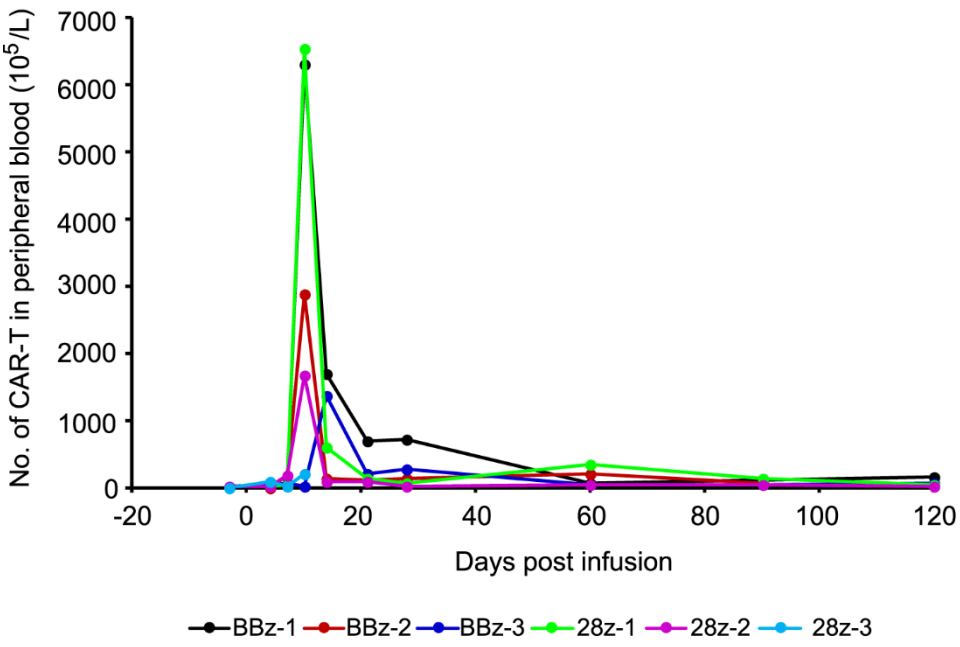
Supplementary Figure 1. The nucleotide sequences of BBz CAR and 28z CAR.



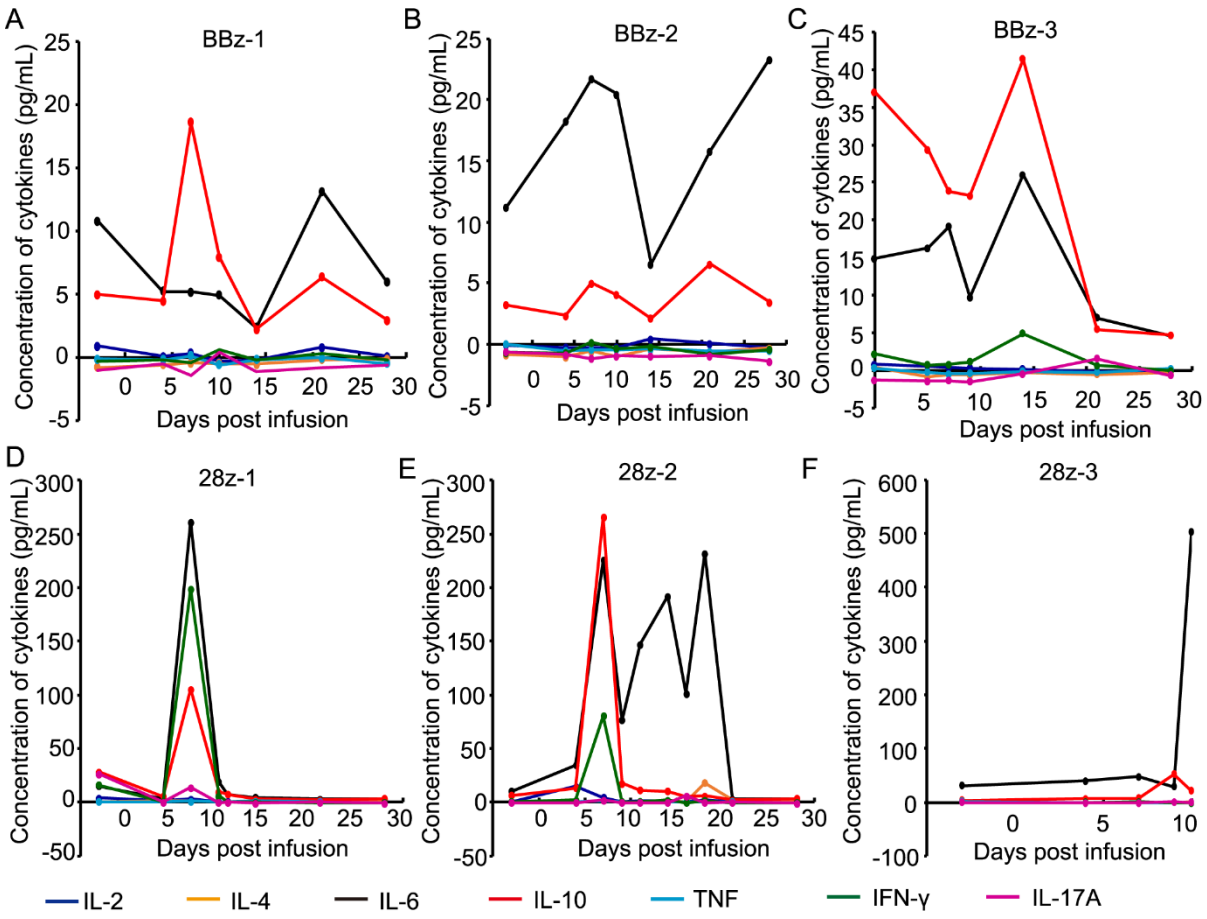
Supplementary Figure 2. Representative dot plots of differentiation status of BBz CAR-T and 28z CAR-T cells by flow cytometry analysis of CD45RA and CD62L expression.



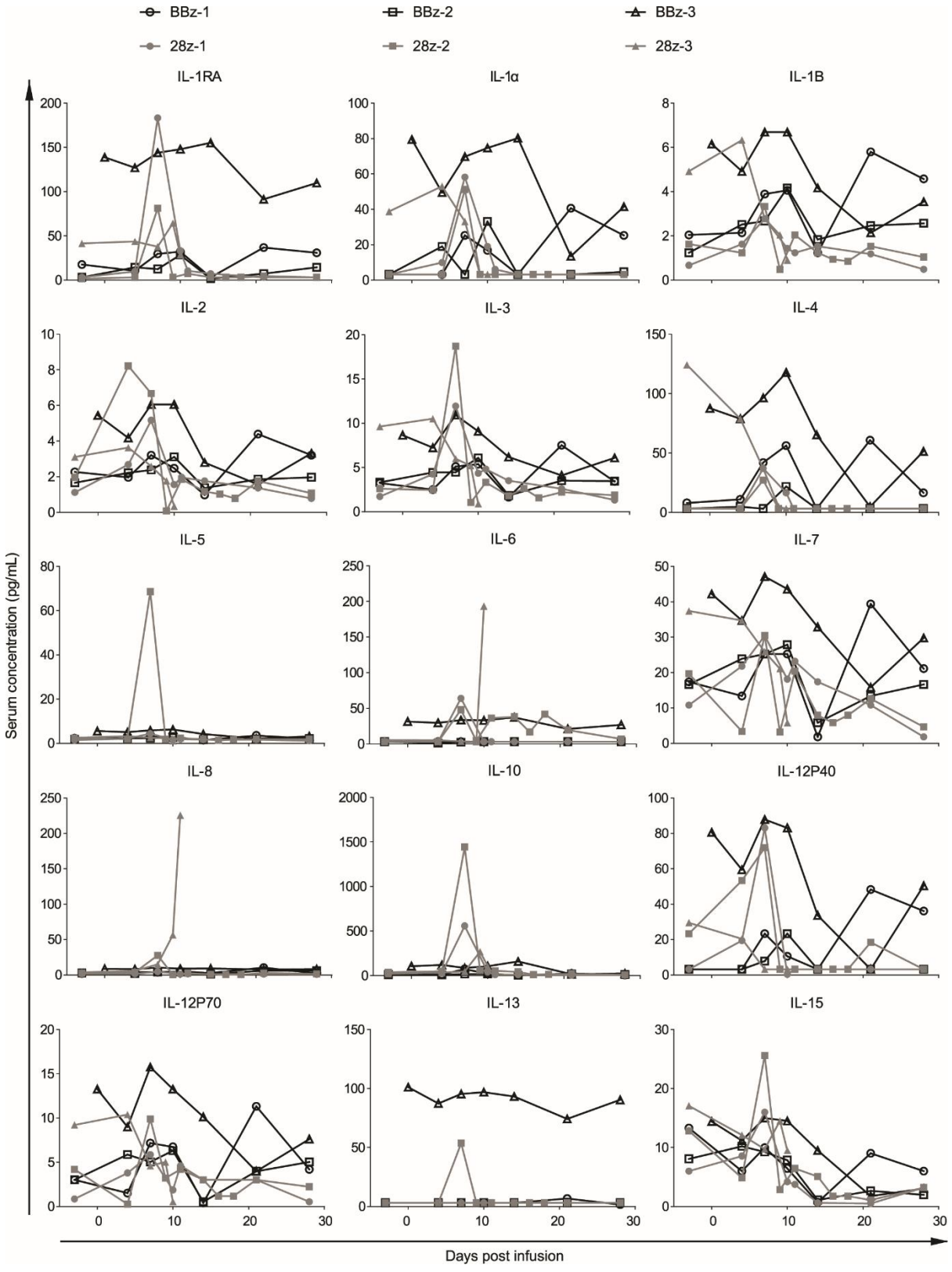
Supplementary Figure 3. The PET-CT scanning of patients before, one month, and three months after infusion of indicated CAR-T cells.



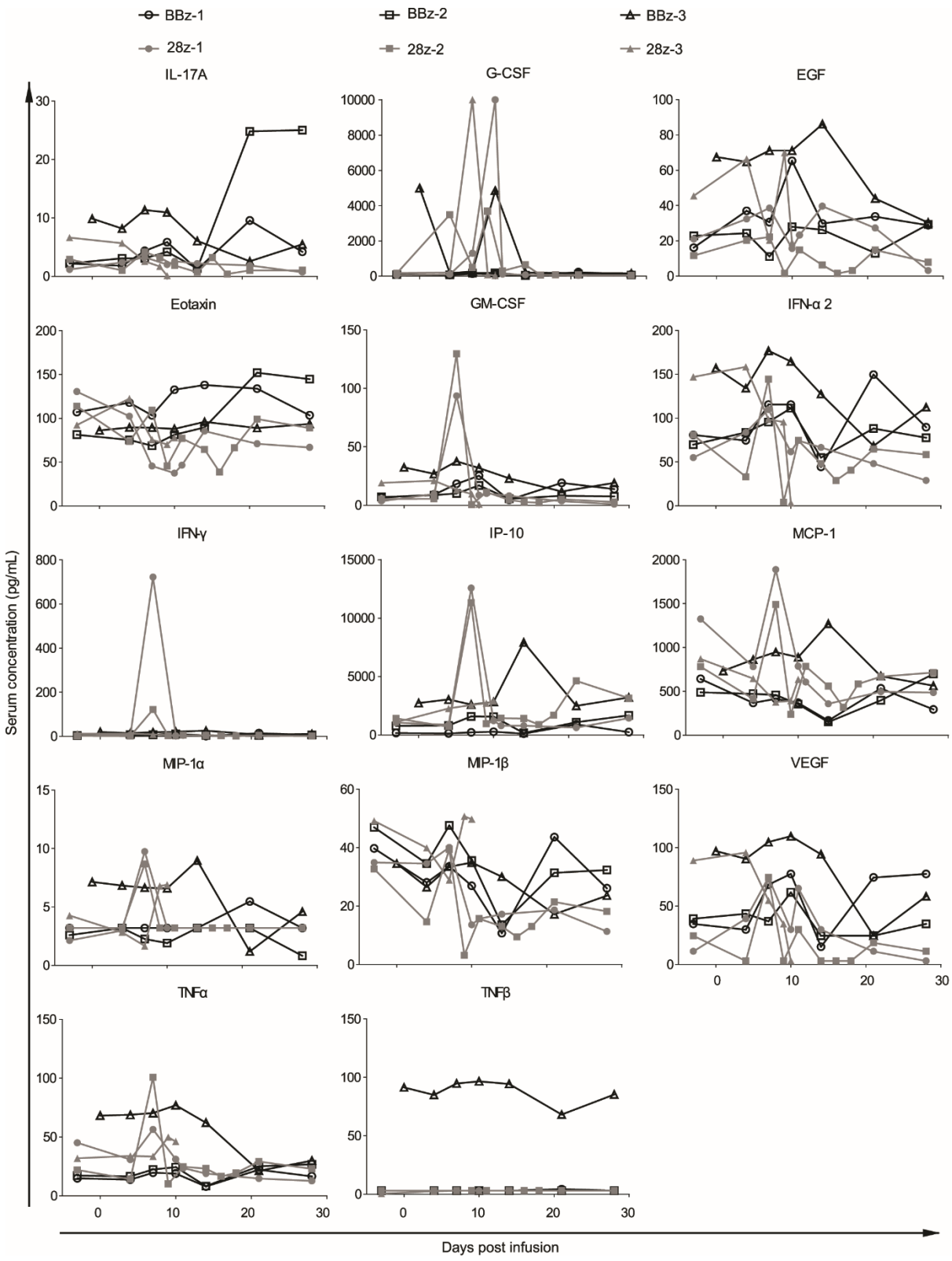
Supplementary Figure 4. The number of CAR-T cells in peripheral blood after patients were infused with low dose of CAR-T cells for different time intervals.



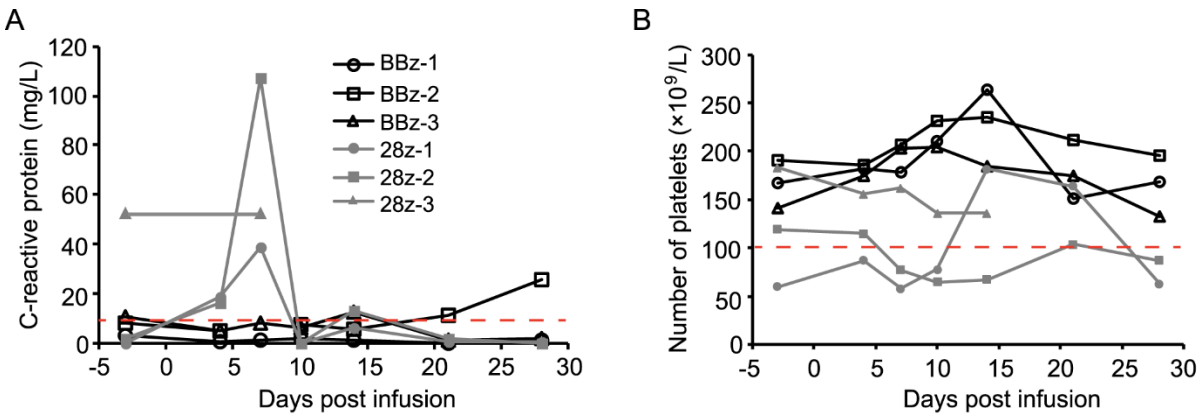
Supplementary Figure 5. (A-F) The expression levels of cytokines (IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A) in peripheral blood were evaluated using cytometric bead array in each patient after infusion with indicated CAR-T cells for different time intervals.



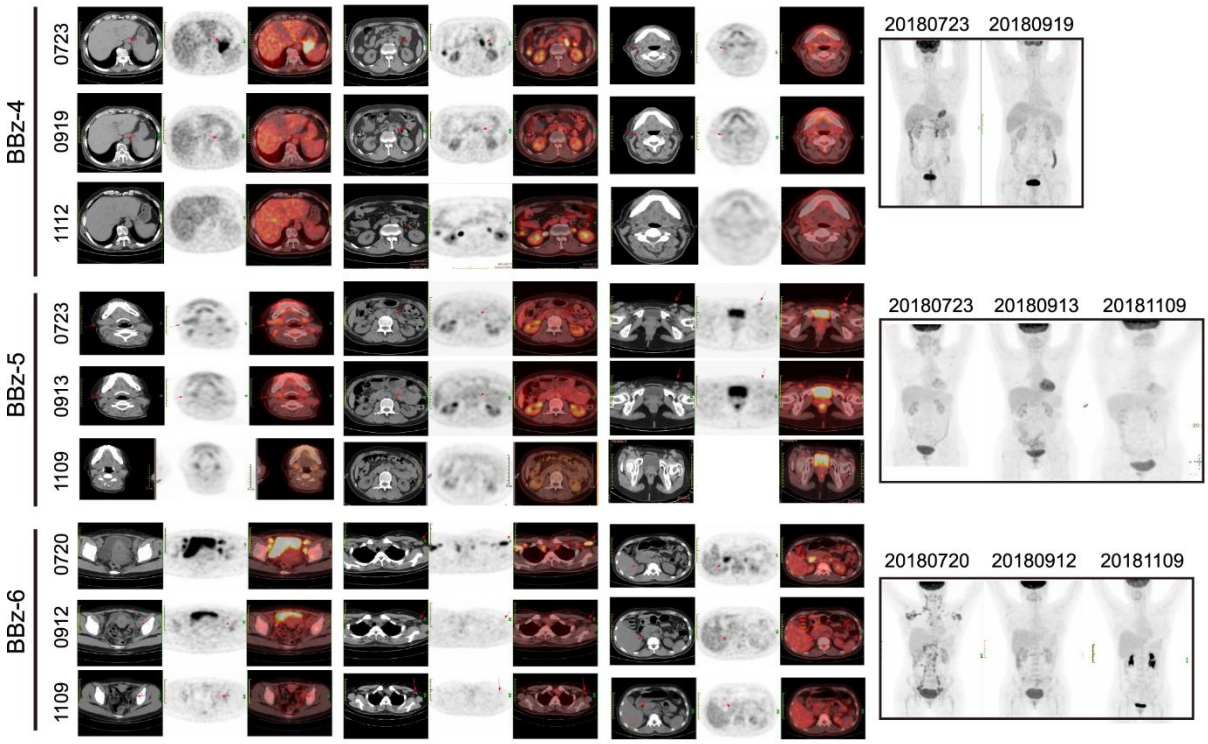
Supplementary Figure 6. The levels of indicated serum interleukins of each patient were evaluated using luminex assay.



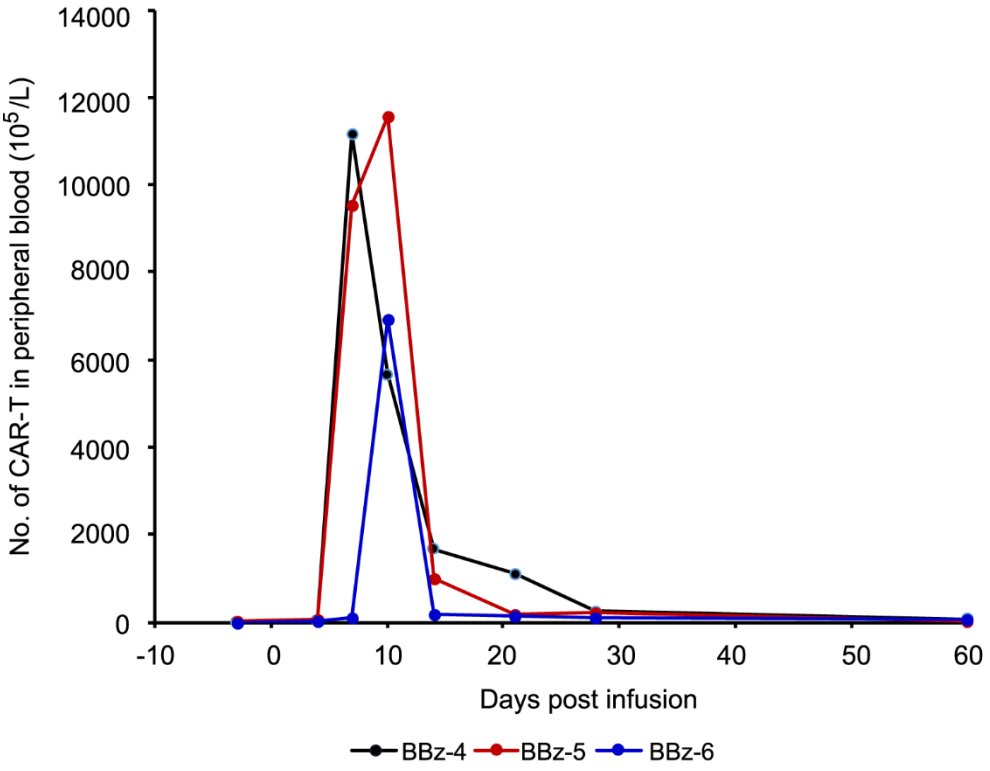
Supplementary Figure 7. The levels of indicated inflammatory cytokines and growth factors in serum of each patient were evaluated using luminex assay.



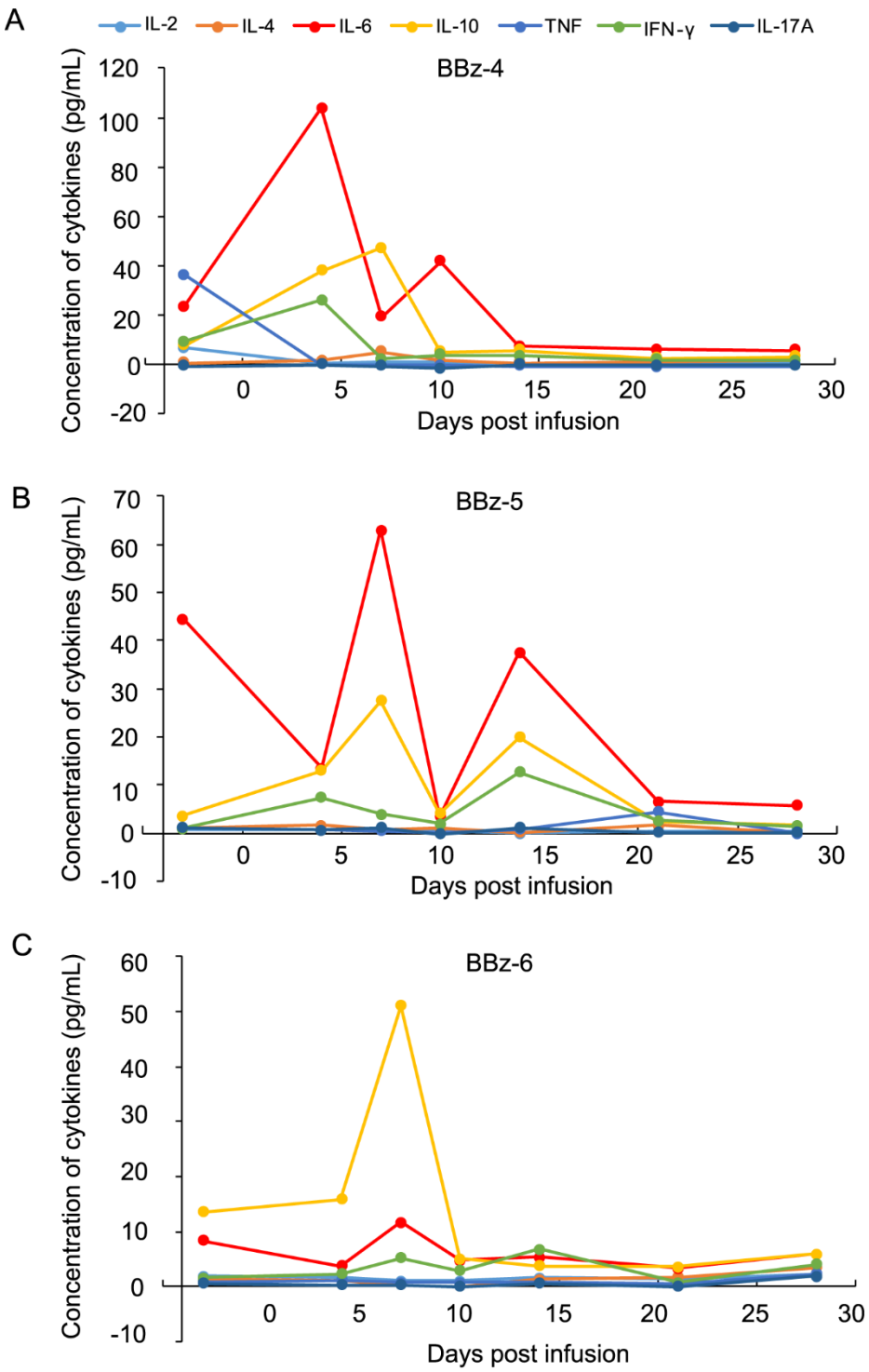
Supplementary Figure 8. Comparison of C-reactive protein level and platelet amounts in patients who were treated with BBz CAR-T or 28z CAR-T cells. (A) The level of C-reactive protein was monitored after patients were treated with indicated CAR-T cells. Red dashed showed the normal level at highest. (B) The number of platelets in patients receiving indicated CAR-T cells was followed up. Red dashed showed the normal level at lowest.



Supplementary Figure 9. The cross-sectional PET-CT images of patients who were infused with an elevated dose of CAR-T and all showed complete response.



Supplementary Figure 10. The number of BBz CAR-T cells in peripheral blood after they were infused to patients at an elevated dose.



Supplementary Figure 11. (A-C) The expression levels of cytokines (IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A) in peripheral blood were evaluated using cytometric bead array in each patient after infusion with an elevated dose of CAR-T cells for different time intervals.

Supplementary Table 1. Detailed chemotherapy regimens for patients before enrollment

Chemotherapy regimen	Components
CHOP	Cyclophosphamide, Doxorubicin, Oncovin, Prednisone
R-CHOP	Rituximab, Cyclophosphamide, Doxorubicin, Oncovin, Prednisone
R-DDP+VP-16+DXM	Rituximab, Cisplatin, Etoposide, Dexamethasone
GEMOX	Gemcitabine, Oxaliplatin
RICE	Rituximab, Ifosfamide, Cisplatin, Etoposide
DICE	Dexamethasone, Ifosfamide, Cisplatin, Etoposide

Supplementary Table 2. Ratio of B cells in the peripheral blood of each patient after CAR-T infusion

Patient No.	Percentage of B cells in peripheral blood after CAR-T infusion (%)						
	Day10	Day 14	Day 21	Day 28	Month 2	Month 3	Month 4
BBz-1	1.31	0.21	0.57	0.04	0.4	1.13	0.2
BBz-2	0.09	0.07	0.02	0.28	1.11	0.8	-
BBz-3	0.36	1.55	0.09	0.32	0.08	0.64	1.54
28z-1	0.32	0.26	0.52	0.23	0.38	63.86	31.51
28z-2	0.24	0	0.18	0.2	0.93	2.46	9.74
28z-3	3.24	-	-	-	-	-	-
BBz-4	0.69	3.04	1.31	0.56	0.28	0.09	0
BBz-5	1.57	0.8	0.19	0.68	3.41	12.62	19.67
BBz-6	1.63	1.25	0.13	0.42	0.11	4.26	7.95

Supplementary Table 3. The concentration of cytokines in peripheral blood and cerebrospinal fluid of patient 28z-2

	IL-2	IL-4	IL-6	IL-10	TNF	IFN-γ	IL-17A
Peripheral blood (pg/mL)	0.87	0.78	146.84	11.44	0.32	0.77	-0.23
Cerebrospinal fluid (pg/mL)	0.91	0.26	313.09	15.11	0.32	-0.61	-0.10

Supplementary Table 4. The blood routine indexes and C-reactive protein level in each patient

Patient No.	Day	Hb (g/L)	RBC (10^{12}/L)	WBC (10^9/L)	LYM (10^9/L)	PMN (10^9/L)	PLT (10^9/L)	CRP (mg/L)
Normal level		120-160	3.5-5.5	4-10	1-5	2-8	100-350	< 8
BBz-1	-3	130	4.6	2.46	0.23	2.01	167	3.3
	4	150	5.29	2.49	0.52	1.57	182	0.8
	7	147	5.15	2.14	0.56	1.01	178	1.6
	10	146	5.1	2.83	1.43	0.89	211	2.2
	14	151	5.21	3.04	1.13	1.37	264	1.4
	21	147	4.98	4.55	0.72	3.12	151	-
	28	151	5.28	4.67	1.02	3.13	169	1.5
	60	149	5.11	4.96	1.24	3.04	221	3.3
	90	147	5	6.33	1.76	3.68	206	2.3
	120	142	4.86	5.48	1.62	3.19	191	1.7
BBz-2	-3	134	4.49	4.09	0.08	3.8	191	8.5
	4	124	4.28	2.77	0.13	2.27	186	5.1
	7	138	4.46	2.01	0.21	1.41	207	-
	10	139	4.73	3.13	0.72	1.86	232	7.6
	14	121	4.07	2.65	0.29	1.9	235	5.4
	21	140	4.75	5.12	0.5	3.97	212	11.4
	28	131	4.45	5.36	0.89	3.78	196	25.9
	42	143	4.91	6.63	1.42	4.45	286	17.6
	60	128	4.36	4.21	0.6	3.16	205	17.6
	90	129	4.59	3.12	0.69	1.9	261	35.7
BBz-3	-3	99	3.56	2.34	1.06	1.1	141	10.4
	4	102	3.53	2.08	0.66	1.02	174	5.3
	7	118	3.95	2.08	0.59	1.04	203	8.5
	10	116	3.97	3.06	0.65	1.68	204	6
	14	122	4.12	2.53	0.72	1.58	184	12.3
	21	129	4.33	2.34	0.93	1	174	1.6
	28	126	4.23	3.69	1.8	1.5	133	1.9
	60	144	4.73	4.07	1.73	1.88	164	2.5
	90	134	4.44	5.19	1.54	3.14	209	42.1
	120	153	4.97	4.5	1.66	2.26	205	3.1
28z-1	-3	133	4.01	3.35	0.13	3.03	60	0.3
	4	122	3.69	2.23	0.21	1.72	87	18.7
	7	113	3.37	3.92	0.23	3.34	58	38.7
	10	106	3.19	8.27	1.58	5.31	78	-
	14	120	3.64	4.62	0.84	2.9	182	6
	21	138	4.03	3.37	0.64	2.39	164	0.5
	28	130	3.86	3.06	0.74	2.01	63	0.7
	60	135	3.83	4.12	1.13	2.49	101	0.7
	90	149	4.27	3.58	0.92	2.24	108	0.4
	120	143	4.07	4.69	0.95	3.47	91	0.6
28z-2	-3	90	2.87	1.34	0.15	1.06	119	1.7
	4	76	2.41	1.21	0.51	0.31	115	16.4
	7	76	2.42	3.54	0.13	3.31	77	107.5
	10	73	2.35	8.17	0.59	7.05	65	-
	14	74	2.41	7.45	0.25	6.76	67	13.1

	21	92	2.85	1.75	0.71	0.75	104	2.2
	28	98	3.07	2.09	1.05	0.71	87	0.2
	60	106	3.07	1.1	0.53	0.41	66	0.1
	90	98	2.88	1.77	0.71	0.77	139	2.7
	120	115	3.36	2.3	0.64	1.41	139	-
28z-3	-3	129	4.12	3.02	0.05	2.84	183	52.8
	4	112	3.66	0.69	0.21	0.45	156	-
	7	119	3.79	4.18	0.25	3.06	162	52.7
	10	120	3.81	8.95	0.19	7.68	136	-
BBz-4	-3	107	3.34	2.2	0.05	2.03	59	4.9
	4	98	3.18	1.4	0.17	1.07	89	77.4
	7	91	2.91	6.47	1.61	4.43	93	74.6
	10	101	3.21	2.63	0.93	1.35	131	31.1
	14	103	3.3	2.27	0.47	1.47	133	4.6
	21	122	3.87	3.85	0.72	2.65	90	0.8
	28	122	3.92	2.67	0.72	1.5	98	1.1
	60	137	4.3	4.72	1.22	2.97	99	1.7
BBz-5	-3	123	3.87	6.46	0.13	95	150	29.8
	4	122	3.28	2.19	0.18	1.57	161	5.9
	7	108	3.33	3.31	1.31	1.6	142	43.8
	10	125	3.94	2.91	1.65	26.8	209	14.6
	14	139	4.38	6.35	0.35	4.97	188	9.3
	21	129	4.03	3.36	0.77	2.21	143	2.3
	28	124	3.78	3.63	1	2.07	218	-
	60	144	4.4	4.52	1.36	2.7	171	0.7
BBz-6	-3	85	3.61	4.68	0.08	4.45	289	1.7
	4	89	3.76	2.61	0.43	1.71	288	0.4
	7	87	3.71	3.28	0.39	2.33	285	0.5
	10	86	3.66	16.19	1.61	82.4	329	0.9
	14	96	4.12	3.93	0.78	2.58	386	1
	21	100	4.52	2.89	1.12	1.54	306	0.5
	28	90	3.81	2	0.79	1	287	-
	60	86	3.96	2.66	1.11	1.2	354	0.6

Abbreviations: Hb, hemoglobin; RBC, red blood cell; WBC, white blood cell; LYM, lymphocyte; PMN, neutrophil; PLT, platelet; CRP, C-reactive protein