



The advantage of chimeric antigen receptor T cell therapy in pediatric acute lymphoblastic leukemia with E2A-HLF fusion gene positivity: a case series

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Abstract: The E2A-HLF fusion gene is a very poor biomarker in acute lymphoblastic leukemia (ALL) because of its high relapse risk, even with the most intensive chemotherapy and hematopoietic stem cell transplantation (HSCT). Here, we analyzed four cases diagnosed with E2A-HLF fusion gene-positive B-ALL and treated with the CCCG-ALL-2015 protocol based on high-risk stratification from Jun 2017 to May 2020 retrospectively. Three cases (Case 1, 2, 3) were insensitive to conventional therapy and inhibitors with high-level MRD on days 19 and 46, but they all achieved complete remission at the molecular level with Chimeric Antigen Receptor (CAR) T cell therapy regardless of primary resistance or recurrence. Although remission was initially achieved for Case 4, chemotherapeutics was not sensitive after recurrence. However, CAR-T cell therapy gave him the chance to obtain complement remission again. Cytokine release syndrome (CRS) with fever, chills, acute kidney injury, hypotension and capillary leak syndrome and CAR-T related encephalopathy syndrome (CRE) with seizures and encephaledema occurred after CAR-T cell therapy, but symptoms disappeared with effective intensive care. Overall, CAR-T cell therapy enabled the patients to achieve complement remission with controllable adverse events. Our results indicated that CAR-T cell therapy is a feasible and effective therapy for patients with E2A-HLF-positive B-ALL and prompted the authors to report these cases.

Keywords: E2A-HLF; pediatric; acute lymphoblastic leukemia (ALL); CAR-T cells therapy; case series

Submitted Oct 05, 2020. Accepted for publication Dec 23, 2020.

doi: 10.21037/tp-20-323

View this article at: <http://dx.doi.org/10.21037/tp-20-323>

Introduction

The incidence of the E2A-HLF fusion gene in ALL is approximately 1% and is caused by the t(17;19)(q22;p13) translocation to form a mutant fusion protein that is thought to play a role in pathogenesis (1,2). Moreover, this gene was identified as a poor prognostic indicator in B-ALL patients (3,4). Glover *et al.* reported a patient who benefited from dasatinib combined with chemotherapy (5). Fischer and Frismantas held the view that venetoclax was an effective inhibitor to cure this disease (6,7). In addition, a report showed that haploidentical transplantation followed

by intensive donor lymphocyte infusions (DLIs) led to remission in a patient (8). However, CAR-T cell therapy applied in this kind of disease has rarely been reported. In this report, we described 4 cases diagnosed with E2A-HLF-positive B-ALL that showed a rapid response to CAR-T cell therapy. We present the following cases in accordance with the AME Case Series Checklist (available at <http://dx.doi.org/10.21037/tp-20-323>).

Methods

All procedures performed in studies involving human

participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patients' parents/guardians. Four patients went to receive CAR-T cell therapy as a clinical trial which was approved by the ethics committee of Children's Hospital of Soochow University [2016004].

This study is a case series. Four cases diagnosed with E2A-HLF fusion gene-positive B-ALL and treated with the CCCG-ALL-2015 protocol (9) based on high-risk stratification were retrospectively collected from Jun 2017 to May 2020 in Children's Hospital of Soochow University.

Results

Case 1 showed a poor response to the standard induction therapy of prednisolone, vincristine, cyclophosphamide, daunorubicin, and L-asparaginase (VDLD) with the CCCG-ALL 2015 protocol as the high-risk group (9). On day 19, the minimal residual disease (MRD) was 1.64×10^{-1} by flow cytometry. After consolidation therapy with cyclophosphamide, cytarabine, and mercaptopurine (CAM), the MRD was still 7.6×10^{-2} on day 46. Then, reinduction therapy was started with vincristine, mitoxantrone, and dexamethasone (MOP). Following this salvage therapy, bone marrow evaluation showed that the MRD was 3.2×10^{-1} . Therefore, CAR-T cell therapy (1×10^7 cells/kg, infusion, 3 times, from himself) was complimented. There were no adverse reactions after the first two infusions, and only a mild fever was observed after the third time. After one week, bone marrow evaluation presented molecular-level remission, with an MRD less than 1×10^{-4} . On day 40 after CAR-T cell therapy, the patient then received 5/10 mismatched haploidentical transplantation from his father on the basis of remission. Seven months later, the patient relapsed, with 65% blasts and 7.68×10^{-1} MRD. CAR-T cell therapy (1×10^7 cells/kg, infusion, from his father) was used again to ease the tumor burden. There were incidences of hypotension, chills, acute kidney injury and increased body temperature after CAR-T cell therapy. Tocilizumab, norepinephrine and continuous renal replacement were used to relieve these symptoms. Ten days later, he achieved remission, and then, umbilical cord blood transplantation was carried out. After successful transplantation, there was a time when molecular remission was achieved, but unfortunately, recurrence occurred again in the later stage, even combined with DLI, venetoclax and dasatinib therapy,

and lung GVHD appeared after transplantation. Finally, the patient died of respiratory failure on day 570 after diagnosis.

Case 2 employed venetoclax orally following VDLD from the CCCG-ALL 2015 protocol. Bone evaluation on days 19 and 46 showed MRD levels of 3.09×10^{-1} and 3.82×10^{-3} , respectively. CAR-T cell therapy (1×10^7 cells/kg, infusion, two doses, from himself) was used for reinduction, replacing any further intensive chemotherapy. No reaction was found following the first dose. Four days later, the second dose was employed, with resulting fever and hand tremble, but the symptoms disappeared with tocilizumab. The bone marrow was evaluated again one week later and showed fusion gene negativity and an MRD less than 1×10^{-4} . On day 60 after CAR-T cell therapy, the patient was given HSCT, and his father was the donor with HLA 7/10 matched. The treatment succeeded, and the patient has survived for 110 days since diagnosis.

Case 3 was treated with the CCCG-ALL-2015 protocol as well. The MRD was 1.69×10^{-2} and 1.04×10^{-2} on days 19 and 46, respectively. The E2A-HLF fusion gene was still positive. The patient was treated with CAR-T cell therapy (1×10^7 cells/kg, infusion, from himself) instead of consolidation chemotherapy. CRS with fever, hypotension and capillary leak syndrome and CRE with seizures and encephaledema occurred after therapy. Symptoms disappeared with effective intensive care. The bone marrow was showed that the patient achieved CR at day 7 after CAR-T cell therapy. To date, he has survived for 240 days since diagnosis.

Case 4 was enrolled in the same protocol. VDLD was conducted two weeks later because of disseminated intravascular coagulation recurrence. The MRD was less than 1×10^{-4} after induction therapy and remained negative after consolidation chemotherapy with CAM and high-dose MTX. Unfortunately, on day 177 of the maintenance treatment phase, although the MRD was less than 1×10^{-4} , the fusion gene turned positive, with elevated blasts. The patient was given reinduction therapy with cyclophosphamide, idarubicin, vindesine and dexamethasone (CIOP) and reconsolidation therapy with CAM. However, the MRD was still at a high level (1.1×10^{-3}). The patient was given CAR-T cell therapy (5×10^6 cells/kg, twice, infusion, from himself), and at day 14, the MRD and fusion gene turned negative again. On day 70 after CAR-T cell therapy, the disease relapsed and failed to be treated with vindesine, idarubicin, cyclophosphamide, L-asparaginase, and dexamethasone (VDCLP). The patient survived for 378 days after CAR-T cell therapy and died of

Table 1 Clinical features of four cases

Case	Age	Sex	Complaint	Blasts	E2A-HLF	Coagulopathy	Hypercalcemia	WBC ($\times 10^9/L$)	Hb (g/L)	Plt ($\times 10^9/L$)	Gene mutation	Outcome
1	3y	Male	Recurrent cough	93%	Positive	Yes	No	32.26	109	96	Negative	Deceased form GVHD
2	14y8m	Male	Repeating nausea and vomiting, bone pain	40%	Positive	No	No	7.66	127	130	NRAS (p Gly12Asp)	Alive
3	2y11m	Male	Mental fatigue and vomiting	89%	Positive	Yes	No	9.49	121	181	Negative	Alive
4	10y4m	Male	Skin bleeding and weakness of lower limbs	92%	Positive	Yes	Yes	11.43	143	51	Negative	Deceased form relapsed disease

WBC, white blood cells; Hb, hemoglobin; Plt, platelet; GVHD, graft-versus-host disease.

resistant disease.

The clinical features of the four cases are summarized in *Table 1*. The pretreatment regimens for CAR-T cell therapy in the 4 cases are illustrated in *Figure 1*. The variation of the E2A-HLF fusion gene and MRD levels are also illustrated in *Figure 2*. *Table S1* shows the changes in cytokines after CAR-T cell therapy and intensive supportive care in these patients.

Discussion

The four cases were diagnosed with E2A-HLF fusion gene-positive B-ALL and treated with the CCGG-ALL-2015 protocol based on high-risk stratification. Overall, 3 of 4 patients did not achieve effective molecular remission with conventional chemotherapy and inhibitors, but they all achieved complete remission at the molecular level with CAR-T cell therapy regardless of primary resistance or recurrence. One patient (Case 3) did not obtain remission by induction and consolidation therapy, but he achieved molecular remission after CAR-T cell therapy, with 4 months of disease-free survival, which indicated the efficacy of CAR-T cell therapy (10). This was the first successful case in which remission was achieved only via this remedy. Another patient (Case 2), who first adopted conventional chemotherapeutics combined with venetoclax, was not cured, which was in contrast with previous studies (6,7,11). Fischer *et al.* reported that such patients were sensitive to anthracyclines, glucocorticoids and venetoclax through drug sensitivity (7). Jason *et al.* also found that dasatinib combined with intravenous chemotherapeutics resulted in a patient achieving 9 years of disease-free survival, which suggested that dasatinib may be beneficial (5). In addition, DLI is regarded as an effective treatment (8). However, for Case 1, although the patient endured all these therapies, unfortunately, he did not obtain a good outcome. Although remission was initially achieved for Case 4, chemotherapeutic therapy was not sensitive after recurrence. However, CAR-T cell therapy gave him the chance to obtain complement remission again.

Currently, CAR-T cell therapy has been successfully applied to treat relapsed/refractory disease (12). However, the use of this therapy in B-ALL with t(17;19)(q22;p13) translocation has rarely been reported. It is well known that this kind of disease has a poor outcome because of its high resistance to chemotherapeutics, inhibitors and even HSCT. Compared to chemotherapy, CAR-T cell therapy has shown remarkable activity against B-ALL, with high

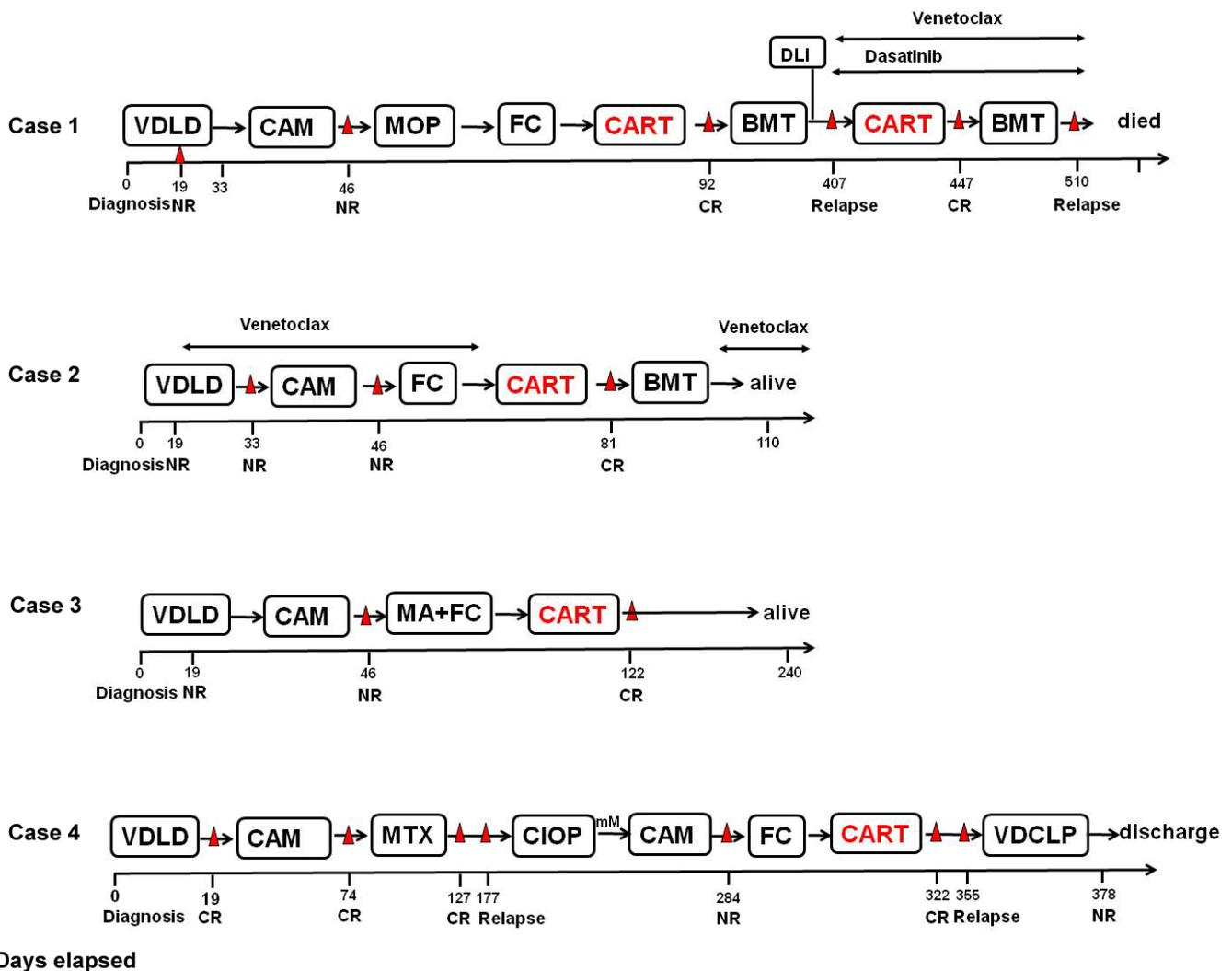


Figure 1 Flow chart of treatment protocol with four cases. VDLL, prednisolone, vincristine, cyclophosphamide, daunorubicin, and L-asparaginase; CAM, cyclophosphamide, cytarabine, and mercaptopurine ; MOP, vincristine, mitoxantrone, dexamethasone; FC, cyclophosphamide, fludarabine; MA+FC, cytarabine, mitoxantrone, cyclophosphamide, fludarabine; CIOP, cyclophosphamide, idarubicin, vindesine, dexamethasone; VDCLP, vindesine, idarubicin, cyclophosphamide, L-asparaginase, dexamethasone; BMT, bone marrow transplant; CART, CAR-T cells therapy; DLI, donor lymphocyte infusions; MTX, methotrexate.

complete response rates and controlled CRS and CRE, which resembled the results of previous reports of adult and pediatric ALL patients (10,13). However, the limitation of this study is that we only have four cases and more samples should be analyzed in further to validate this conclusion.

Conclusions

CAR-T cell therapy used for E2A-HLF fusion gene-positive ALL proved to be a promising therapy, and this targeted immune therapy should be considered in such patients.

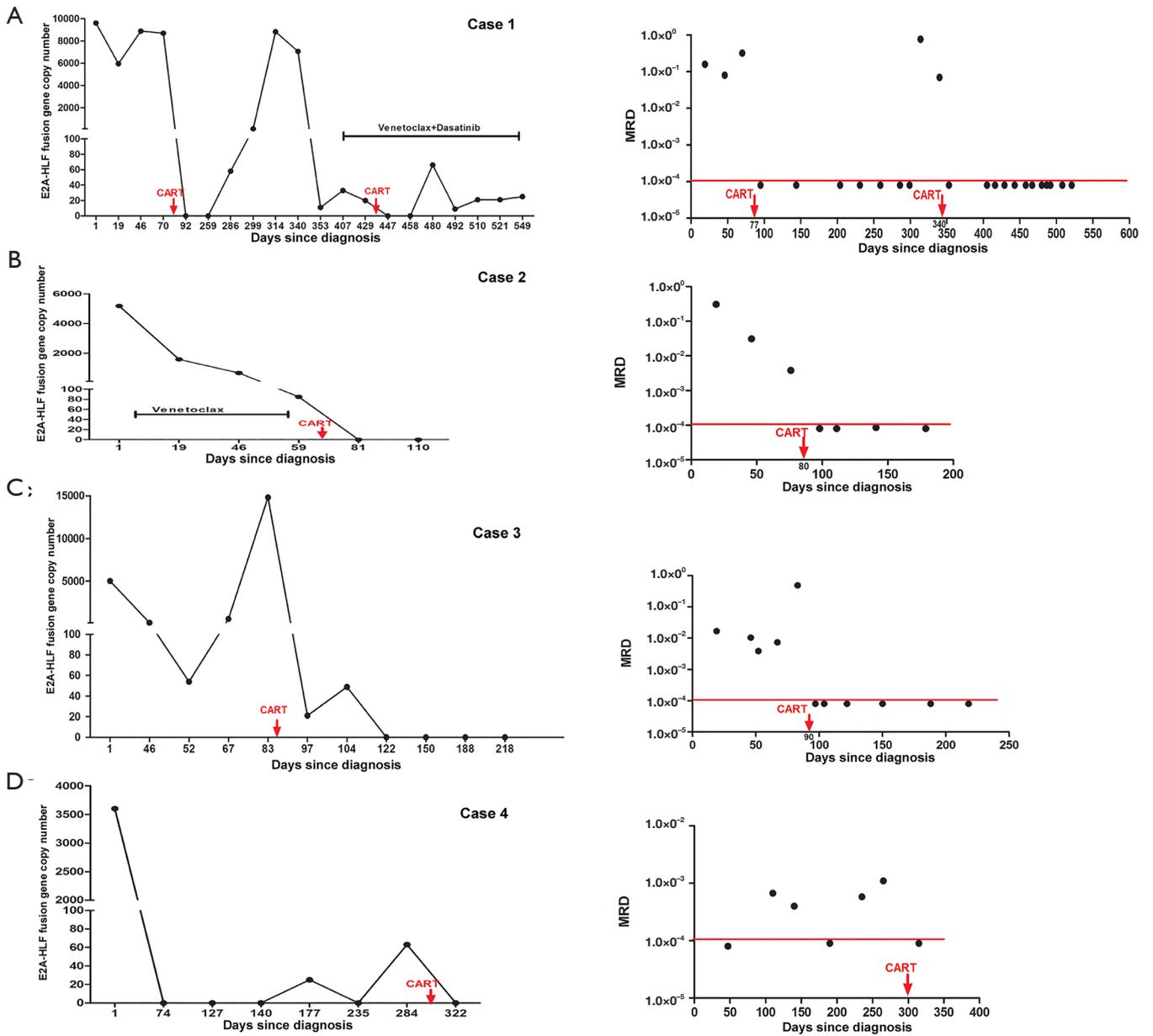


Figure 2 Variation of E2A-HLF fusion gene and MRD in four patients. CART, Chimeric Antigen Receptor (CAR) T cell therapy; MRD, minimal residual disease.

Acknowledgments

Funding: Suzhou Science and Technology Development Project (SYS 201757) and Natural science Foundation of Jiangsu Higher Education Institutions of China (18KJB320022).

Footnote

Reporting Checklist: The authors have completed the AME Case Series Checklist. Available at <http://dx.doi.org/10.21037/tp-20-323>

Peer Review File: Available at <http://dx.doi.org/10.21037/tp-20-323>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tp-20-323>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patients' parents/guardians.

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Cite this article as: Wu S, Lu J, Su D, Yang F, Zhang Y, Hu S. The advantage of chimeric antigen receptor T cell therapy in pediatric acute lymphoblastic leukemia with E2A-HLF fusion gene positivity: a case series. *Transl Pediatr* 2021;10(3):686-691. doi: 10.21037/tp-20-323

Table S1 Change of cytokines after CAR-T therapy and the intensively supportive care in these patients

			IL-2 (pg/ml)	IL-4 (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF α (pg/ml)	IFN (pg/ml)	IL-17A (pg/ml)	Toci	GC	Supportive therapy	Adverse events		
Case1	First	d0	0	1.3	6.7	5.2	0	0	0				fever		
		d1	3.5	3.1	37.5	6.6	4	10	0						
		d5	5.9	0	23.6	5.2	2.7	2.8	0						
		d6	3.8	0	49.8	10.6	0	4.7	0				fever		
		d7												fever	
		d8	1.8	0	19.2	4.2	2.5	2.6	0						
		d9	1.3	4.5	4.5	3.9	2.8	0	0						
		Second	d0	1.2	0	14.7	0.6	2.6	4.3	0					fever
			d1	7.5	2.7	27.5	7.5	3.7	10.6	4					fever,chills
	d2		0	2.3	4.6	1.9	0	3.3	0			NA		fever	
	d3		3.8	5.8	15.7	4.2	4.8	10.5	2	80mg		albumin furosemide		fever	
	d4		7.36	5	3713.44	76.02	1.65	761.34	4.46	160mg		NA		fever, hypotension	
	d5		8.9	17.8	875.5	6.4	4.2	46.7	0			CRRT, red blood cells IVIG		fever,AKI	
	Case2	First	d6	6.7	22	316	2.2	3.7	10.4	0			CRRT	fever,AKI	
			d7	4.3	12.6	218.6	3.2	2.6	12.8	0			CRRT	fever,AKI	
d8			3.3	8.3	256.2	1.7	2.9	16.3	0				edema of the face and eyelids		
Second		d9	0.5	3.2	143	13.5	0	38.4	5.1						
		d1	3.1	<2.5	9.3	25.7	<2.5	5.4	<10						
		d2	<2.5	<2.5	9.1	33.9	2.9	6.5	10.7						
Case3	First	d3	<2.5	<2.5	5.4	24.1	<2.5	3.3	<10						
		d4	<2.5	<2.5	15.8	71	<2.5	3.8	<10					fever, cephalalgia, hands tremble	
		d5	3.1	<2.5	113.4	302.1	<2.5	48.7	<10	320mg				fever, cephalalgia	
	Second	d6	<2.5	<2.5	2984.2	420.8	2	336.9	<10	160mg				fever	
		d7	<2.5	<2.5	192.5	33.9	2.5	4.1	11.9						
		d0	<2.5	<2.5	21.3	8.6	<2.5	<2.5	<10					fever	
		d1	11.9	<2.5	46	12.1	<2.5	19.8	<10	80mg	dex(3mg)	UTI NA		fever, eyelid swelling, oliguria,weight gain,chills	
d2	8.7	3.3	127.4	25.2	6.4	126.8	<10			UTI,albumin MAP		fever, facial edema, peritoneal and pleural effusion			
d3	9.4	4.3	1650.2	81.8	2.9	306.6	13.4	80mg	dex(3mg)	UTI, albumin fib		fever, eyelid edema, slight pericardial effusion			
d4	12.2	14.2	5968.3	290.6	9.8	1637.4	44.3	160mg	dex(3mg) medrol (80mg)	UTI,albumin, fib,NA phenobarbitone		seizure hytotension			
Case4	First	d5	<2.5	<2.5	1200.9	145.8	<2.5	1274.4	14.9	80mg		UTI,albumin plasma and fib		fever	
		d6	2.9	4.2	552.5	18.6	3.7	67.1	20			UTI,albumin, fib PCC			
		d7	<2.5	<2.5	359.8	8.5	<2.5	12.8	<10			UTI			
		d3	5.4	4.0	23	5.3	5.8	21.2	16.1						
		d4	N/A	N/A	N/A	N/A	N/A	N/A	N/A					fever	
		d5	4.8	4.2	8.8	5.0	9.6	52.8	15					fever,chills	
d6	3.2	4.6	28.9	4.3	3.6	0	0					fever			
d7	3	3.5	13.7	3.6	2.7	0	0								
d8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A			plasma		coagulopathy		

CAR-T therapy, Chimeric Antigen Receptor (CAR) T cell therapy; UTI, ulinastatin; dex, dexamethasone; Toci, tocilizumab; GC, glucocorticoids; NA, norepinephrine; PCC, prothrombin complex concentrate; CRRT, continuous renal replacement therapy; AKI, acute kidney injury; medrol, methylprednisolone; N/A, no detection; IVIG, intravenous immunoglobulin.