Efficacy and safety of cilta-cel in patients with progressive MM after exposure to other BCMA-targeting agents

SUPPLEMENTARY APPENDIX

Supplementary Methods

CAR transgene assessment

The pharmacokinetics (PK) of cilta-cel were assessed by measuring transgene CAR copies at multiple time points after cilta-cel administration. The validated qPCR method was performed to quantitate CAR transgene constructs and report relative to total genomic DNA present in samples. Separate aliquots were drawn to serve as template for the CAR transgene and for the apolipoprotein B (ApoB) assays. The CAR transgene assay used primers and probe specific for the transgene of cilta-cel to specifically amplify the transgene DNA present in the incurred sample. The ApoB assay employed primers and probe specifically for the natural human ApoB sequence in the genomic DNA. The 2 qPCR assays were performed independently, and threshold cycle values used to determine absolute copy numbers (per microgram genomic DNA) present in the incurred sample.

Serum BCMA assessment

A ligand-binding assay was used to quantify sBCMA in a homogeneous electrochemiluminescent immunoassay (ECLIA). sBCMA calibrators, sBCMA quality controls, and human serum samples containing sBCMA were diluted in assay buffer and then again in a master mix containing a biotin-labeled anti-human BCMA antibody and a Sulfo-Tag (Ruthenium) labeled anti-human BCMA antibody. Samples were incubated with master mix and then added to a streptavidin-Meso Scale Discovery (MSD) plate. These were incubated and then washed and appropriate read buffer was added and then detected using the MSD platform.

anti-CAR antibody assessment

A sensitive method on the MSD platform was developed and validated to screen, confirm, and titer anti-drug antibody (ADA) to recombinant extracellular BCMA-binding domain of cilta-cel in human serum samples. A screening method was used to detect potentially positive ADA to cilta-cel in human serum samples, and a specificity (confirmation) method was used to determine whether potentially positive samples were either ADA positive or ADA negative. Positive samples were evaluated in a titration method to provide a quasi-quantitative assessment of ADA reactivity in serum samples. The serum samples were incubated with a biotin-tagged recombinant protein version of the extracellular, anti-BCMA domain of the cilta-cel CAR. Magnetic streptavidin-coated beads were added to the samples and incubated to capture anti-cilta-cel antibodies. The beads were collected by a magnetic plate and washed. The beads were

treated with acid to elute the anti-cilta-cel antibodies, then treated with buffer to neutralize the pH. The samples were then treated with the recombinant biotin-tagged anti-BCMA extracellular CAR protein and these were added to a streptavidin-coated MSD plate and incubated. Sulfo-Tagged anti-immunoglobulin (Ig)G and IgM detection reagents were then added to the samples. Samples were treated with read buffer and read on the MSD Sector Imager. Signal was generated by the detection reagents binding to the anti-cilta-cel antibodies bound to the recombinant version of the CAR.

Serum cytokines assessment

Thirteen cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor [TNF]-α, IL-5, IL-17A, IL-13, IL-2 receptor alpha [IL-2RA], and IL-12/IL-23p40) were measured using validated multiplex sandwich immunoassays on the MSD platform. The samples were added to the MULTI-SPOT plates pre-coated with capture antibodies and incubated at room temperature. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface. After wash, a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SOLFO-TAGTM) was added to the plate followed by another incubation period. An MSD buffer that creates the appropriate chemical environment for electrochemiluminescence was added after the final wash. The plate was then loaded into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte presented in the sample) and provides a quantitative measure of each analyte in the sample.

Supplemental Table 1. Responses by treatment history with BCMA-targeting agent

Patient	Best response to anti-BCMA agent	Duration of last anti-BCMA agent (days)	Time from last anti-BCMA agent to apheresis (days)	Time from last anti-BCMA agent to cilta-cel infusion (days)	Received anti-BCMA as last line (Y/N)	Responder to cilta-cel (Y/N)	Best confirmed response to cilta-cel[a]	Cause of death (if applicable)
ADC-1	sCR	527	895	944	N	N	SD	
ADC-2[b]	PD	1	594	679	N	Y	sCR	
ADC-3	SD	21	695	749	N	Y	VGPR	PD
ADC-4	VGPR	277	182	271	N	Y	CR	AE: Covid-19 pneumonia
ADC-5[c]	PD	63	57	99	Y	N	PD	
ADC-6	PD	23	161	243	N	Y	CR	
ADC-7[d]	NE	1	85	147	N	Y	CR	
ADC-8 [b]	PD	64	26	62	Y	Y	CR	
ADC-9	SD	22	139	180	N	Y	VGPR	
ADC-10	PD	22	49	177	Y	N	SD	
ADC-11	PD	44	128	210	N	Y	VGPR	
ADC-12	SD	64	56	116	Y	N	PD	PD
ADC-13	VGPR	54	40	95	N	N	PD	PD
BsAb-1	SD	127	280	329	N	Y	CR	
BsAb-2	SD	36	281	325	N	Y	VGPR	
BsAb-3	VGPR	71	161	227	Y	Y	PR	AE: Covid-19 pneumonia
BsAb-4	CR	260	77	119	N	N	SD	
BsAb-5	PD	15	251	307	N	N	MR	AE: Subarachnoid hemorrhage
BsAb-6[b]	PD	23	28	84	Y	Y	VGPR	
BsAb-7	SD	130	84	124	N	N	NE	AE: *C difficile* colitis

[a]Assessed by validated computerized algorithm.
[b]Patient had baseline high-risk cytogenetics (del17p).
[c]Patient received 2 different ADCs.
[d]Patient received BsAb first and ADC later.

Supplemental Table 2. Bridging therapy, measurable disease type, and tumor burden change from screening to cilta-cel infusion

Patient	Bridging therapy received	Bridging therapy regimen and duration (days)	Measurable disease type at screening	Tumor burden at screening[a]	Change in tumor burden at cilta-cel infusion[b]
ADC-1	Yes	Bortezomib (4), Cisplatin (4), Cyclophosphamide (4), Dexamethasone (4), Doxorubicin (4), Etoposide (4)	FLC	536.4 mg/L	Decreased >50%
ADC-2	Yes	Bortezomib (15), Cyclophosphamide (5), Daratumumab (8), Dexamethasone (20), Doxorubicin (5), Selinexor (15)	FLC	154.98 mg/L	Decreased ≤50%
ADC-3	Yes	Cisplatin (1), Cyclophosphamide (1), Doxorubicin (1), Etoposide (1), Methylprednisolone (1)	FLC	240.29 mg/L	Increased ≥25%
ADC-4	Yes	Methylprednisolone (1)	FLC	2080.89 mg/L	Decreased >50%
ADC-5	Yes	Bortezomib (15)	Urine only	0.2111 g/24h	Not evaluable
ADC-6	Yes	Cisplatin (4), Cyclophosphamide (4), Dexamethasone (4), Etoposide (4)	FLC	367.12 mg/L	Decreased >50%
ADC-7	Yes	Methylprednisolone (1)	Serum and urine	0.6265 g/24h	Increased ≥25%
ADC-8	Yes	Cyclophosphamide (8), Methylprednisolone (16)	Serum only	20 g/L	Did not change
ADC-9	No		FLC	1198.86 mg/L	Not evaluable
ADC-10	Yes	Cyclophosphamide (73), Dexamethasone (76), Pomalidomide (73)	Serum and urine	29 g/L	Decreased >50%
ADC-11	Yes	Dexamethasone (50), Selinexor (50)	Serum and urine	48 g/L	Increased <25%

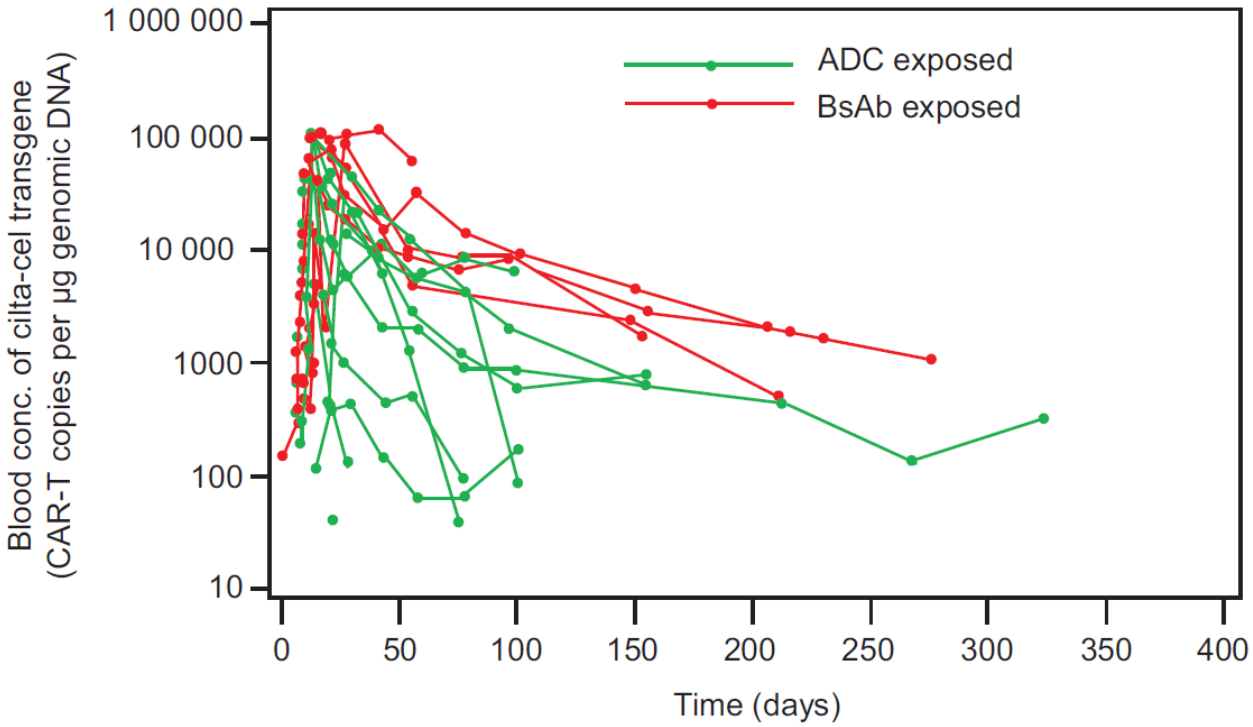
ADC-12	Yes	Bortezomib (29), Dexamethasone (37), Selinexor (36)	NE	8.74 g/L	Not evaluable
ADC-13	Yes	Cisplatin (4), Cyclophosphamide (4), Dexamethasone (4), Doxorubicin (4), Etoposide (4)	FLC	120.5 mg/L	Not evaluable
BsAb-1	No		Urine only	0.4841 g/24h	Increased ≥25%
BsAb-2	Yes	Carfilzomib (22), Dexamethasone (29), Pomalidomide (29), Selinexor (22)	Urine only	1.3232 g/24h	Increased ≥25%
BsAb-3	Yes	Cisplatin (4), Cyclophosphamide (4), Dexamethasone (4), Etoposide (4)	Serum only	22 g/L	Increased ≥25%
BsAb-4	Yes	Carfilzomib (22), Cyclophosphamide (22), Dexamethasone (29)	FLC	432.5 mg/L	Increased <25%
BsAb-5	Yes	Dexamethasone (32), Pomalidomide (40)	Serum and urine	0.2623 g/24h	Decreased ≤50%
BsAb-6	Yes	Cisplatin (4), Cyclophosphamide (4), Doxorubicin (4), Etoposide (4)	Serum only	12 g/L	Increased ≥25%
BsAb-7	Yes	Carfilzomib (12), Cyclophosphamide (1), Dexamethasone (17), Selinexor (12)	Urine only	0.9827 g/24h	Increased ≥25%

[a]Presented as either serum or urine value, whichever showed greatest burden.

[b]Measured as myeloma paraprotein/light chain.

FLC, free light chain.

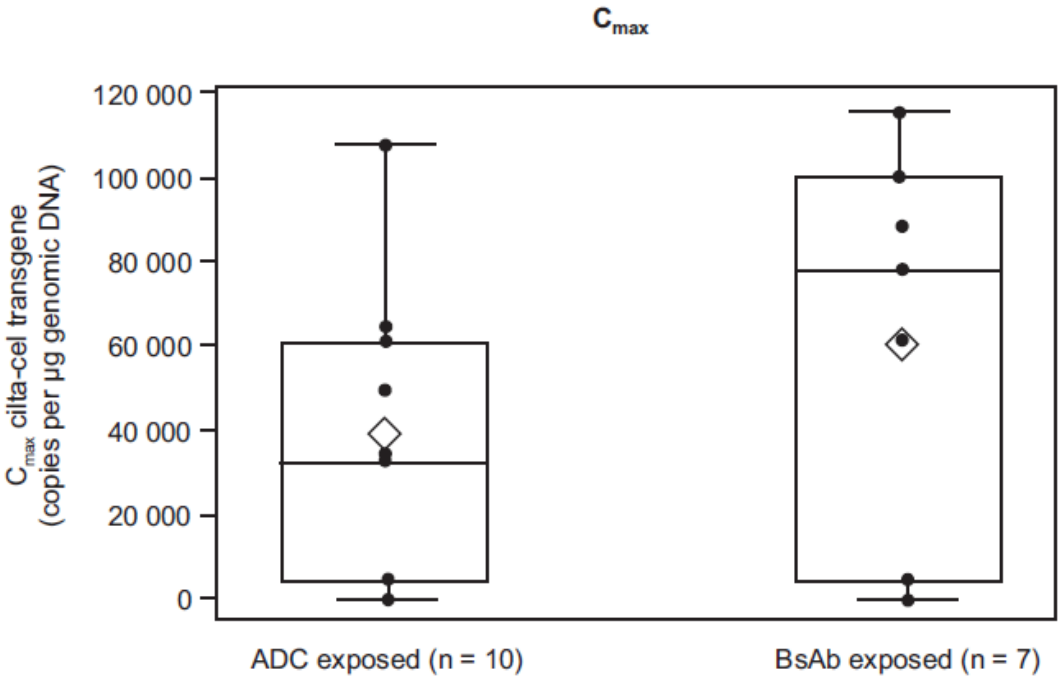
Supplemental Figure 1. Blood concentration-time curves of cilta-cel transgene in individual patients.



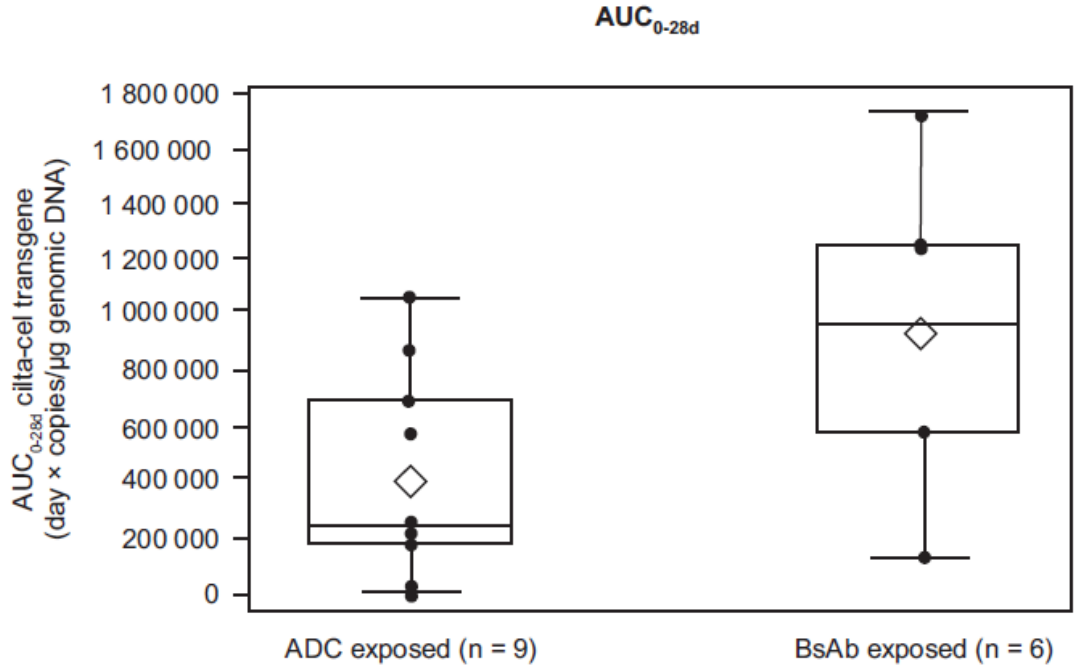
Supplemental Figure 2. Blood pharmacokinetic parameters of cilta-cel transgene.

In the ADC group, 3 patients were missing transgene PK data and 4 were missing $AUC_{0\text{-}28d}$ data.

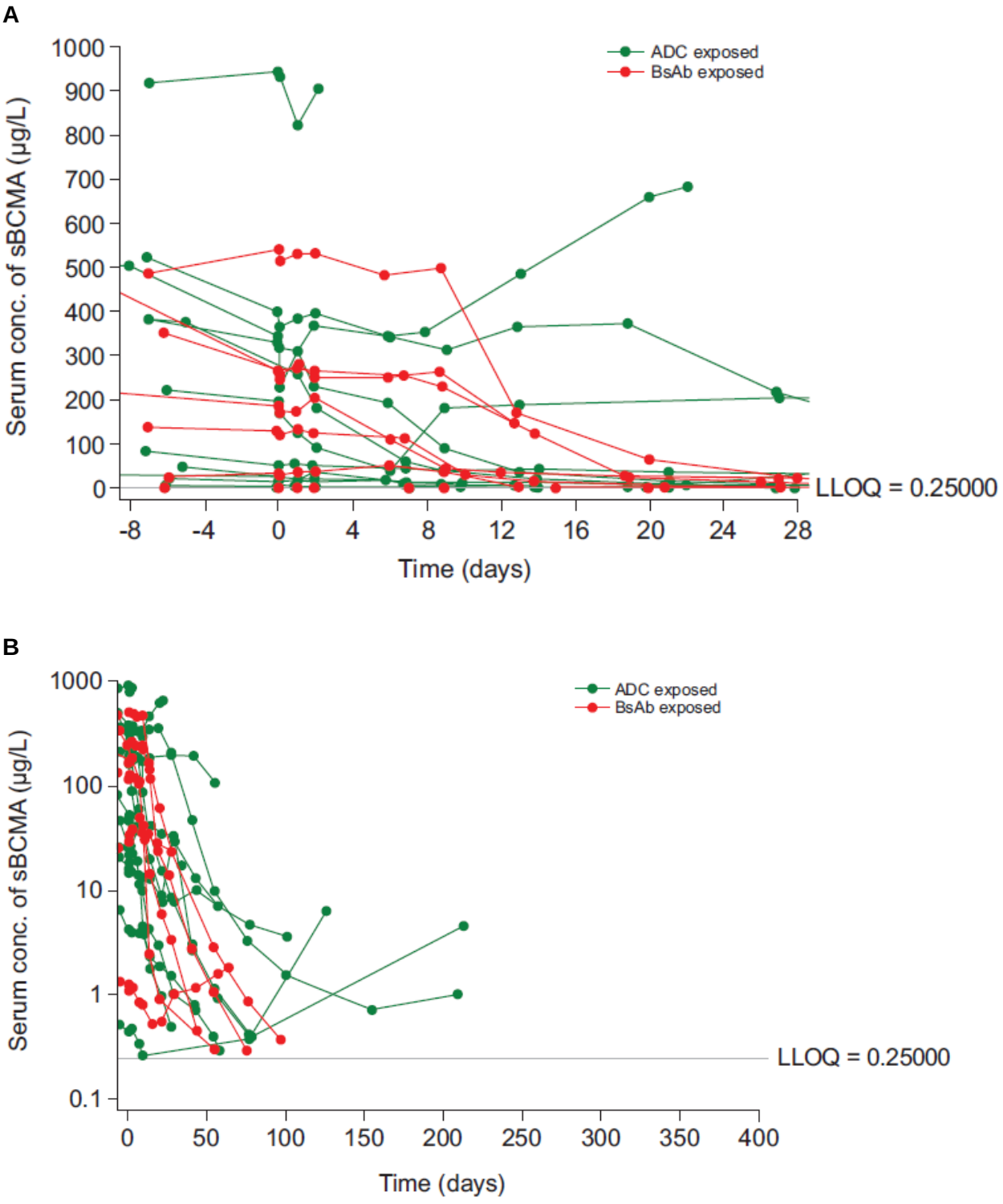
A



B



Supplemental Figure 3. Serum BCMA concentration over time in individual patients.



LLOQ indicates lower limit of quantification; and sBCMA, serum BCMA.