Supplementary Appendix

Supplement to: Mailankody S, Devlin SM, Landa J, et al. GPRC5D-targeted CAR T cells for myeloma. N Engl J Med 2022;387:1196-206. DOI: 10.1056/NEJMoa2209900

This appendix has been provided by the authors to give readers additional information about the work.

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MCARH109 manufacturing

Patient apheresis product was collected and CD4+ and CD8+ cells were selected using CD4+ Microbeads and CD8+ Microbeads respectively via CliniMACS-plus (Miltenyi). Briefly, on day 0, cryopreserved CD4+ and CD8+ selected cells were thawed, washed, combined at CD4+CD3+ and CD8+CD3+ cells at 1 to 1 ratio, and activated by paramagnetic Dynabeads CD3/28 (Dynabeads ClinEx Vivo CD3/CD28, Invitrogen) at a bead to T cell ratio of 1:1. One day post activation, cells were transduced with lentiviral vector via spinoculation in PermaLife cell bags (OriGen Biomedical) at room temperature. Transduced cells were inoculated in Xuri W25 bioreactor (Cytivia) with perfusion mode approximately 48hr post transduction. Medium used for the culture was X-VIVO 15 (Lonza) supplemented with heat-inactivated human AB Serum (Gemini), Glutamax, IL2, IL7, and IL15 (CellGenix). At the end of process, the paramagnetic Dynabeads CD3/28 were removed using Dynal ClinExVIVO MPC magnet (Invitrogen). Cells were washed, formulated, and cryopreserved using control-rate freezer (ThermoFisher).

MCARH109 expansion and persistence after MCARH109 infusion

Serial evaluation of expansion and persistence of MCARH109 in the peripheral blood was done using real time PCR. DNA was extracted from 200uL whole blood on the Promega Maxwell® RSC Instrument using the Promega Maxwell RSC A54500 kit and eluted in 50uL. 5uL of DNA extract were amplified on the Applied Biosystems Via 7 instrument with primers and probe specific to the MCARH109 construct. The results were reported in vector copies per mL of blood. The sensitivity cut-off is 50 vector copies per mL of blood.

Multiparametric flow cytometric analysis of MCARH109

Cells were washed and resuspended in PBS, then incubated with Human TruStain FcX Fc receptor blocking solution (Biolegend) and Live/DEAD Fixable Blue Dead Cell Stain (Invitrogen) according to the manufacturers' specifications for 20 minutes at room temperature (RT), protected from light. The cells were washed once in Flow Wash Buffer (FWB; RPMI 1640 no phenol red + 4% FBS +0.01% sodium-azide) and incubated with the antibody mix for 20 minutes at RT in the dark in the presence of Brilliant Staining Buffer (BD). The cells were washed, resuspended in 0.5% paraformaldehyde/PBS, and immediately acquired using a Cytek Aurora 5L flow cytometer (Cytek). The optimal concentration of all antibodies used in the study was defined by titration. Further information about the antibodies can be found in Supplementary Table 1. Analysis was performed with FlowJo v10.8.1.

Bone marrow and plasmacytoma GPRC5D expression

We evaluated the GPRC5D antigen, using immunohistochemical method. We used a commercially available antibody, clone 6D9 (ab55044; Abcam, Waltham, MA) at 1:1K dilution (1.0 µg/mL). Staining was performed on the Leica Bond-3 auto staining system (Leica, Buffalo Grove, IL), using heat-based antigen retrieval, a high pH buffer solution (AR9640; Leica, Bond Epitope Retrieval Solution 2, 30 minutes), 30-minute primary incubation time, and a polymer detection system (DS9800; Leica, Bond Polymer Refine Detection).

Morphologic features and CD138 immunohistochemical staining were used as a control for percentage and distribution of plasma cells in submitted slides. The percentage and distribution were then correlated to assess GPRC5D expression on CD138 positive cells. A scoring system was developed to assess GPRC5D percentage and intensity. Intensity was score as weak, moderate, and strong; scores ranged from 1-3. 1 is the score for faint membranous, often incomplete staining and/or weak intracytoplasmic staining. 2 is the score for moderate intensity staining, complete membranous staining, with or without cytoplasmic staining. 3 is the score reserved for strong complete membranous staining, often observed with cytoplasmic immunoreactivity and Golgi accentuation. Any, as low as <5%, expression of GPRC5D on CD138 positive cells was reported in 10% increments. A binary final score was given (0 &1). Any intensity/percentage of GPRC5D was given a final score of 1. A final score of 0 denoted negative GPRC5D on all CD138 positive cells examined.

Results of CSF analysis in the two patients with cerebellar toxicity

Patient 011 had the procedure on 11/19/21 and the results are as follows- Total cells: 18; RBC: 1/microL; nucleated cells: 1/microL; lymphocytes: 89%; protein: 28 mg/DL; glucose: 61 mg/dL; meningitis/encephalitis PCR panel negative; gram stain and bacterial culture: negative; encephalopathy-autoimmune panel: negative; adenovirus: negative; Lyme disease antibodies: negative; Cytology: negative for malignant cells. Patient 016 has the procedure on 10/25/21 and the results are as follows- Total cells counted: 81; RBC: 243/microL; nucleated cells: <1/microL; protein: 19 mg/dL; glucose: 46 mg/dL; meningitis/encephalitis PCR panel negative; gram stain and bacterial culture: negative; JC virus: negative; EBV PCR: negative; cytology: no malignant cells; flow cytometry: no abnormal plasma cells detected. Patient 016 had a repeat procedure on 2/15/22 and the results are as follows- flow cytometry: 1. No abnormal plasma cell population detected. Virtually no plasma cell seen. No evidence of an abnormal plasma cell population is identified by flow cytometry; 2. No abnormal mature T cell population detected. Virtually no T cell seen. No immunophenotypic evidence of T-cell lymphoma is identified. Protein: 21 mg/dL; glucose: 55 mg/dL; Total cells counted: 14; RBC: 5/microL; nucleated cells:

1/microL. On 2/25/22 we also assessed for CAR+ cells in the CSF by flow cytometry and detected a 3 cells that were CAR+ (total events: 194,240; cells: 31,702; CD45+ cells: 138; CD3+ cells: 24; CD3+CAR+ cells: 3; CD3+/CAR+/CD4+: 3).

Figure S1: Consort Diagram for the trial

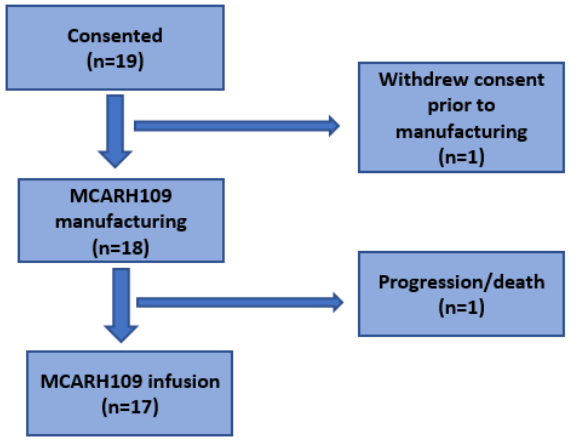


Figure S2A: Timeline of clinical events for patient 011

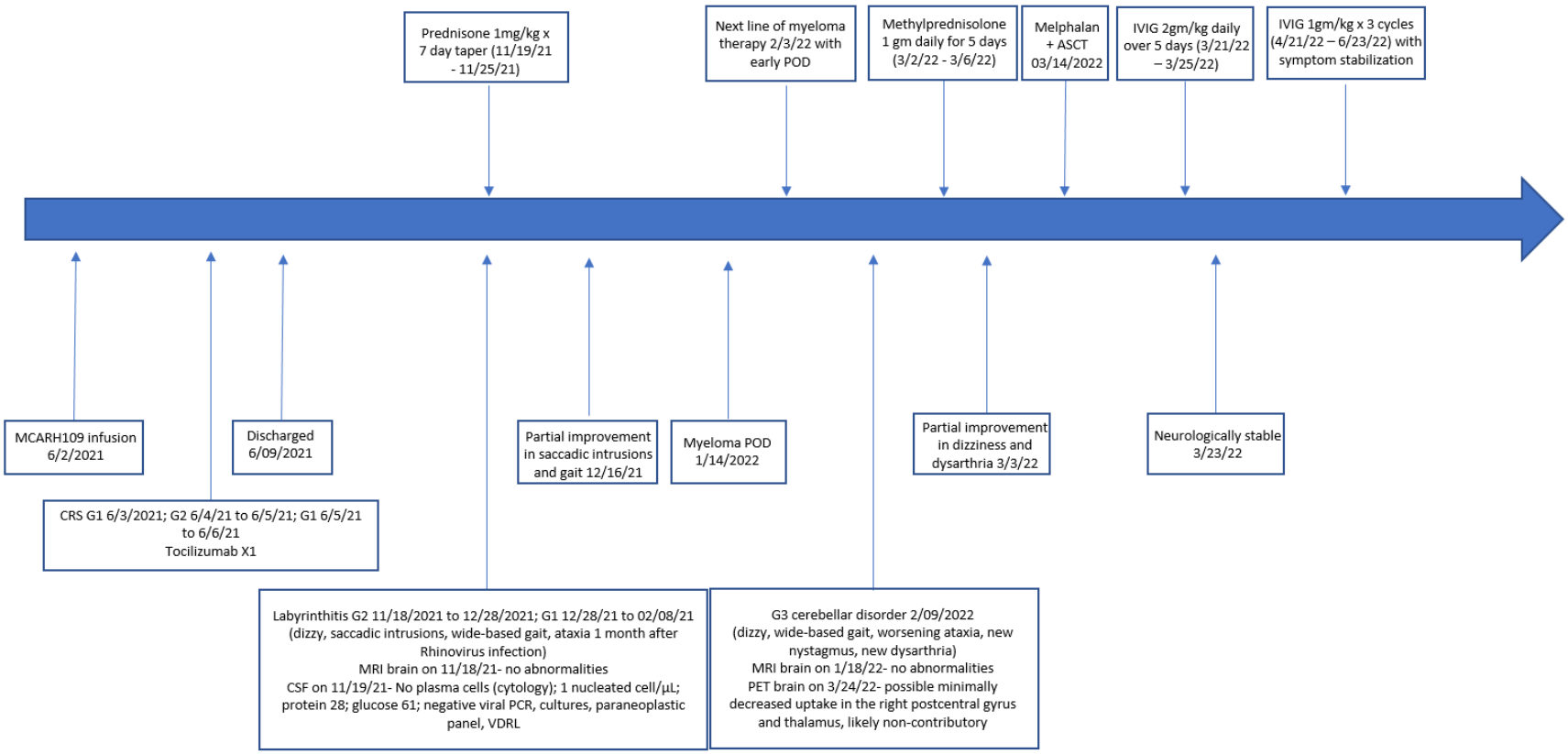


Figure S2B: Timeline of clinical events for patient 016

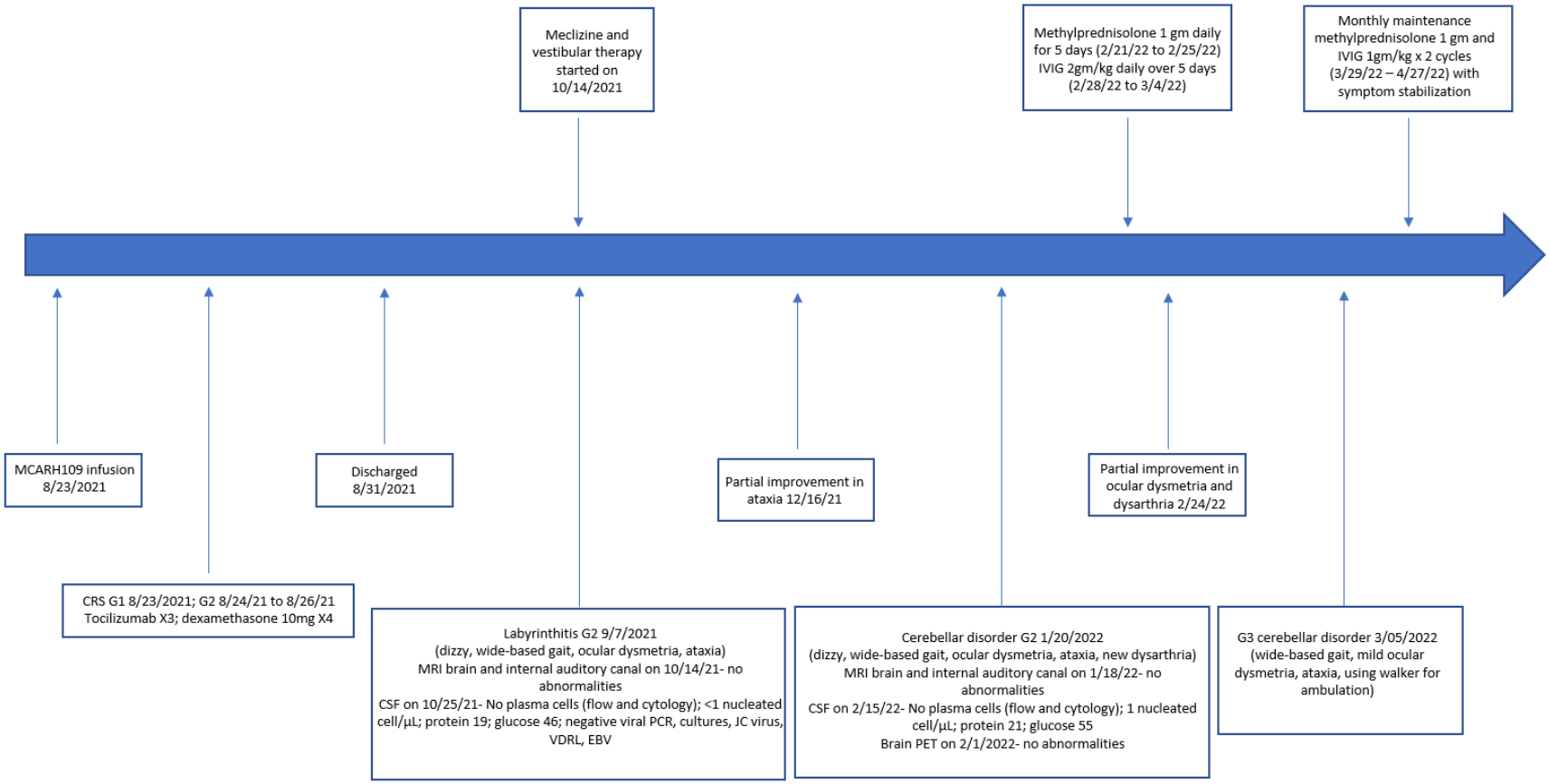
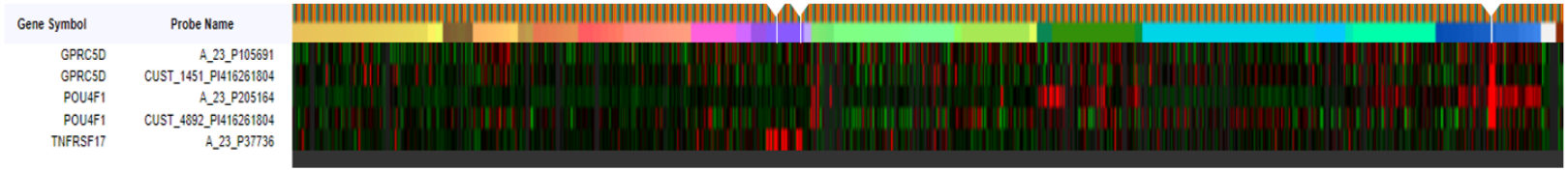
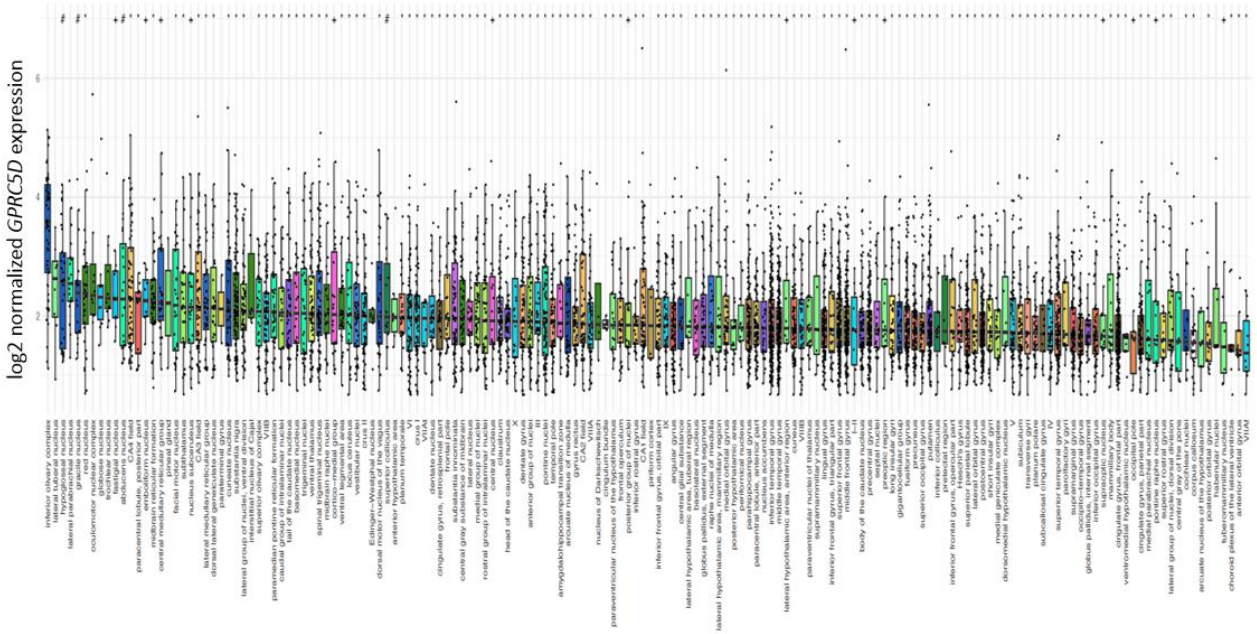


Figure S3A: Microarray expression data (z-score) of the Allen Brain Atlas for two probes for *GPRC5D* from six healthy brain donors grouped according to the brain region demonstrating high *GPRC5D* expression in the inferior olivary nucleus (IOC). Shown for comparison, expression data from *POU4F1* (*BRN3A*; two probes) the most differentially expressed gene in the IOC relative to other brain regions; and *TNFRSF17* (*BCMA*; single probe) predominantly expressed in the caudate nucleus and putamen.



White carrots indicate the caudate, putamen and IOC, from left to right, respectively. Image credit: Allen Institute for Brain Science; available from: human.brain-map.org. Coloring: top row represents 6 individual donors; second row represents brain regions and are consistent across the figure.

Figure S3B: Quantitative representation of the Allen Brain Atlas *GPRC5D* microarray expression data for two probes with boxplots showing normalized expression (log2) across all six donors for all brain sub-structures, grouping all with left/right delineation.



Boxplots represent median, 25th and 75th percentiles and whiskers up to 1.5 * interquartile range beyond the boxes. The normalized expression (log2) across all six donors is shown for all brain sub-structures, with grouping all with left/right delineation (N =7404 observations). Significance levels (* ≤ .001, ⁺ ≤ .01, # ≤ .05) are from unpaired two-sample, two-sided Welch t-tests comparing expression in the IOC against each other structure and have been corrected for multiple testing using a Bonferroni correction.

Figure S3C: Brain sub-structures classified within the myelencephalon graphed separately for enhanced visualization (same data and analysis as supplementary Figure 3B)

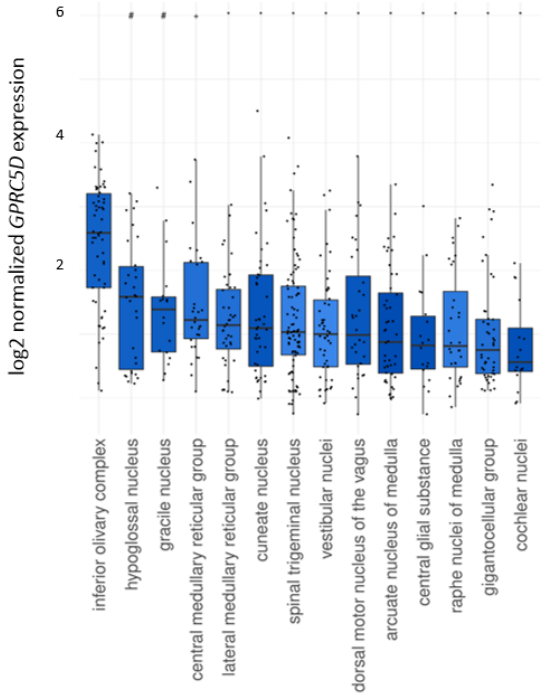
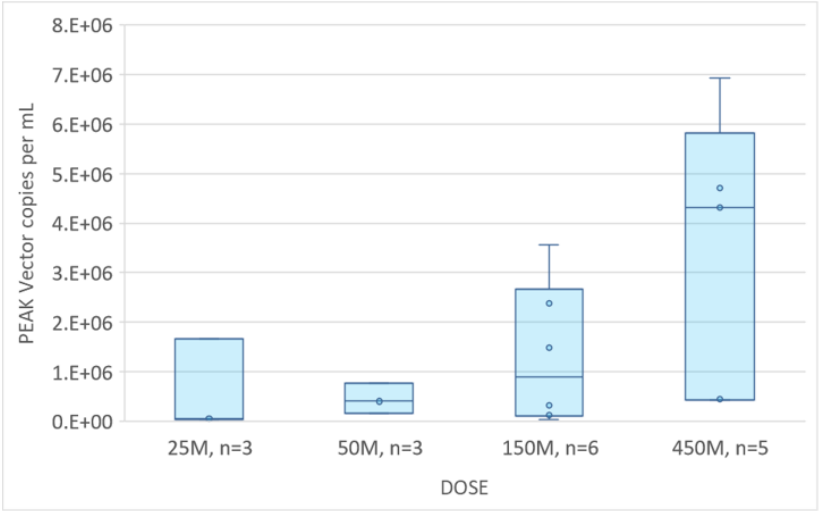
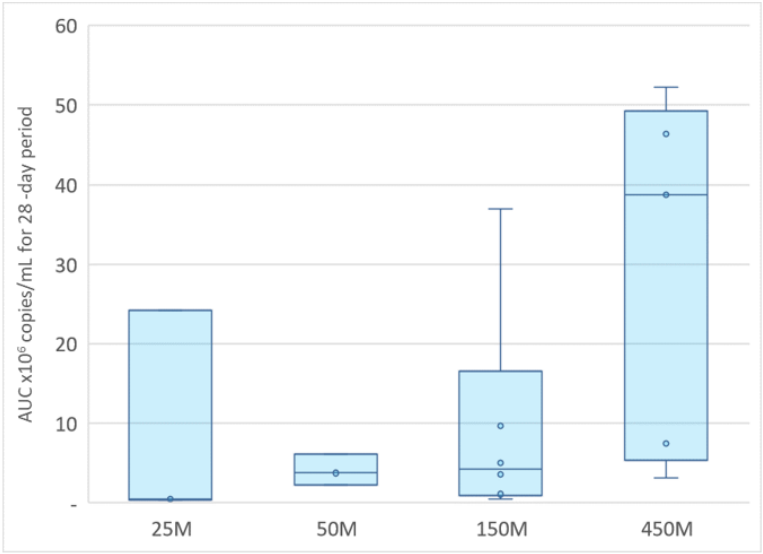


Figure S4A: Peak MCARH109 expansion in the peripheral blood across dose levels



Peak MCARH109 expansion was measured by peak vector copies per mL. Dose levels are as follows: 25M = 25 x 10^6, 50M = 50 x 10^6, 150M = 150 x 10^6 and 450M = 450 x 10^6 CAR T-cells

Figure S4B: Median area under the curve over the first 28 days post-MCARH109 infusion (AUC_{0-28} days)



Dose levels are as follows: 25M = 25 x 10^6, 50M = 50 x 10^6, 150M = 150 x 10^6 and 450M = 450 x 10^6 CAR T-cells

Table S1: Baseline characteristics, response to MCARH109 infusion, and GPR5CD expression by immunohistochemistry pre-infusion and at time of relapse

		Baseline characteristics				Response to MARCH109			Pre-infusion GPRC5D expression[$]			Relapse	GPRC5D expression at relapse[$]		
ID	Dose level	Myeloma subtype	Measurable disease	High risk cytogenetics[†]	EMP	Best overall response	% reduction in EMP	BM MRD negativity	Y/N	% expression on plasma cells	Staining intensity		Y/N	% expression on plasma cells	Staining Intensity
1	1	IgA kappa	Serum M-spike	Y	Y	VGPR	99.1%	Y	Y	100%	3+	Y	Y	10%	1+
2	1	Free lambda	EMP	Y	Y	PD	N/A	N	Y	80%	1+	Y	N	0%	N/A
3	1	Free kappa	EMP	Y	Y	SD	31.3%	Y	Y	100%	3+	Y	Y	70%	1+ & 3+[‡]
4	2	IgG lambda	Free light chains	Y	N	sCR	N/A	Y	Y	90%	1+	N	N/A	N/A	N/A
5	2	Free lambda	Free light chains	Y	N	sCR	N/A	N	Y	100%	1+	Y	Y**	30%	1+
6	2	Free lambda	Free light chains	N	Y	PR	89.1%	Y	Y	90%	1+ & 3+[§]	N	N/A	N/A	N/A
7	3	IgG kappa	Serum M-spike	N	N	SD	N/A	N	Y	100%	2+	Y	Y	80%	1+
9	3	Non-secretory	BM plasma cells	N	N	SD	N/A	N	N	0%	N/A	N	N/A[##]	N/A	N/A
10	3	IgG kappa	Serum M-spike	Y	Y	PR	91%	Y	Y	5%	1+	Y	N	0%	N/A
15	3	Free kappa	EMP	Y	Y	sCR	100%	Y	Y	80%	2+	Y	N	0%	N/A
18	3	IgG kappa	Serum M-spike	Y	Y	VGPR	100%	Y	Y	70%	2+	N	N/A	N/A	N/A
19	3	Free lambda	Free light chains	N	Y	SD	N/A	N	Y	< 5%	1+	Y	N/A	N/A	N/A
11	4	Free lambda	Free light chains	Y	N	sCR	N/A	N	Y	50%	1+	Y	N	0%	N/A
13	4	Free kappa	Urine M-spike	Y	N	VGPR	N/A	N	N	0%	N/A	Y	N	0%	N/A
14	4	IgA kappa	Serum M-spike	Y	N	sCR	N/A	N	Y	100%	1+	N	N/A	N/A	N/A
16	4	IgA lambda	Serum M-spike	Y	N	sCR	N/A	Y	Y	80%	3+	N	N/A	N/A	N/A
17	4	IgG kappa	Serum M-spike	Y	N	VGPR	N/A	Y	Y	90%	2+	N	N/A	N/A	N/A

EMP, extramedullary plasmacytoma; BM, bone marrow; VGPR, very good partial response; PD progressive disease; SD, stable disease; (s)CR, (stringent) complete response; PR, partial response; MRD, minimal residual disease; Y/N, yes/no; N/A, not available. †High-risk cytogenetic abnormalities included the following: 1q amplification, del (17p), t (4;14), or t (14;16); ** Bone marrow biopsy obtained several months after progression of disease; ## Biopsy could not be obtained at progression; $ Pre-and post-infusion samples included either bone marrow biopsies or

plasmacytomas. § There was bimodal GPRC5D staining intensity of 1+ and 3+ within the plasma cells. ‡ There was bimodal GPRC5D staining intensity of 1+ (in 60% of plasma cells) and 3+ (in 10% of plasma cells). The IDs for patients with an objective response are highlighted in red.

Table S2: Pre-infusion characteristics including release criteria for MCARH109

Parameters	% CAR	% CD3	Vector copy number per cell	Viability post-thaw	Average number of beads	Cytotoxic T-Lymphocytes lysis	CAR+CD4+ CD8− CAR T helper cells (% of CD3+)	CAR+CD4−CD8+ cytotoxic CART cells (% of CD3+)	CAR+CD45RA+CCR7+ Naïve CART cells (% of CAR+)	CAR+CD45RA−CCR7+ central memory CART cells (% of CAR+)	CAR+CD45RA−CCR7− effector memory CART cells (% of CAR+)	CAR+CD45RA+CCR7− terminally differentiated effector CART cells (% of CAR+)
Method	Flow Cytometry	Flow Cytometry	Real-time PCR	Acridine Orange/Propidium Iodide	Microscopy	Cytotoxic T-Lymphocytes lysis of target cells	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry	Flow cytometry
Specification	≥ 4%	≥ 80%	≤ 4 copies per cell	≥ 65%	≤ 100 beads / $3x10^6$ cells	< 1 CAR: target ratio for 50% lysis	NA	NA	NA	NA	NA	NA
1	43.7	99.2	1.2	79.1	1.67	0.523	69.8	26.5	72.6	3.0	2.4	24.4
2	31.7	99.3	0.9	81.0	3.33	0.228	48.7	30.9	13.6	3.0	2.0	83.3
3	40.2	99.0	1.1	83.1	3.33	0.328	36.4	23.1	11.7	8.7	2.0	78.9
4	39.8	96.1	1.4	81.9	0.00	0.480	34.4	25.1	3.7	2.4	1.4	93.9
5	46.6	97.6	1.7	70.8	3.33	0.353	52.3	35.1	17.2	3.6	2.4	78.2
6	29.6	99.8	1.4	79.7	1.67	0.523	29.7	26.1	19.5	6.6	5.4	72.4
7	47.5	97.4	1.7	84.4	5.00	0.063	45.8	51.3	17.1	4.6	3.7	75.6
9	48.5	99.8	1.5	83.2	0.00	0.231	35.2	26.4	3.5	2.3	3.2	94.2
10	12.6	99.4	0.3	83.8	1.67	0.224	32.7	4.54	0.0	0.4	1.1	99.0
15	41.6	99.1	1.1	80.7	9.99	0.242	29.8	35.1	10.5	5.5	5.5	83.0
18	42.8	99.7	1.1	83.6	1.67	0.366	31.5	17.6	4.0	3.1	3.2	92.9
19	57.1	98.9	1.6	81.3	0.00	0.130	29.6	46.8	13.1	5.6	5.4	80.9
11	36.0	97.6	1.5	81.3	1.67	0.152	52.3	40	6.2	4.5	4.5	87.6
13	55.5	99.6	2.1	84.1	0.00	0.127	36.4	37.9	2.2	1.7	1.4	95.9
14	43.5	96.0	1.2	82.0	0.00	0.273	41.2	33	8.1	4.1	4.3	87.1
16	59.1	99.9	1.6	74.6	3.33	0.299	35.2	35.3	6.2	1.8	1.3	91.7
17	43.9	97.5	1.3	83.7	0.00	0.027	23.6	45.5	0.0	1.9	1.2	98.1

CAR, chimeric antigen receptor; PCR, polymerase chain reaction.

Table S3: Prior multiple myeloma treatment history for patients treated with MCARH109

Patient ID	Line #	Treatment Regimen	Start date	Stop date	Best response	Date of best response	Date of progressive disease
1	1	KRd → Mel 200 ASCT → R	11-06-2015	08-2017	VGPR	12-18-2015	N/A
	2	Elo-Rd	08-16-2017	01-17-2018	SD	-	02-12-2018
	3	Dara-d	02-28-2018	04-18-2018	SD	-	04-17-2018
	4	KCd	05-16-2018	12-14-2018	VGPR	07-09-2018	12-2018
	5	KRd	01-23-2019	11-20-2019	SD	-	11-01-2019
	6	DCEP	12-03-2019	12-08-2019	SD	-	N/A
	7	V-DT-PACE → Mel140 ASCT → KCd-T	01-03-2020	07-2020	VGPR	03-17-2020	07-23-2020
	8	DCEP	08-03-2020	08-07-2020	PD	-	08-31-2020
		Leukapheresis	09-15-2020	09-15-2020			
		Bridging therapy:				-	
		Bendamustine-Vd	09-16-2020	09-16-2020	PD		10-06-2020
		MARCH109 infusion	10-22-2020	10-22-2020			
2	1	VCd → KRd	06-19-2019	07-05-2019	SD	-	N/A
	2	VRd	08-29-2019	12-13-2019	PR	09-19-2019	12-23-2019
	3	Mel200 ASCT	01-15-2020	01-17-2020	PD	-	04-02-2020
	4	Dara-Rd	04-20-2020	05-04-2020	PD	-	05-08-2020
	5	V-DCEP	05-15-2020	06-09-2020	PR	07-07-2020	07-15-2020
	6	T + KCd	07-22-2020	09-05-2020	SD	-	08-21-2020
		Leukapheresis	09-22-2020	09-22-2020			
		Bridging therapy:					
		Elo-Pd	09-25-2020	10-07-2020	PD	-	10-07-2020
		V-P-DCEP	10-10-2020	10-14-2020	PD	-	10-28-2020
		MARCH109 infusion	11-24-2020	11-24-2021			
3	1	KRd → Mel200 ASCT	06-01-2016	11-07-2016	sCR	08-10-2016	08-16-2018

	2	Dara-Rd	09-07-2018	09-12-2019	SD	-	09-20-2019
	3	KPd	10-02-2019	10-17-2019	PD	-	10-16-2019
	4	Vd-PACE (without cisplatin) → Mel200 ASCT → Elo-Pd	11-01-2019	04-16-2020	PR	02-04-2020	04-16-2020
	5	**Belantamab**	05-20-2020	08-13-2020	PR	06-05-2020	08-30-2020
	6	V-DCEP	09-16-2020	09-20-2020	SD	-	-
		Leukapheresis	10-19-2020	10-19-2020			
		Bridging therapy:					
		V-DCEP	10-22-2020	10-26-2020	PD	-	11-09-2020
		Mel40	11-11-2020	11-11-2020	PD	-	12-08-2020
		MARCH109 infusion	12-23-2020	12-23-2020			
4	1	VCd	03-06-2014	04-15-2014	PR	N/A	N/A
	2	VRd → Mel200 ASCT → R	04-15-2014	08-2015	sCR	N/A	08-2015
	3	KRd	08-20-2015	09-2015	SD	-	N/A
	4	KCd	11-11-2015	02-02-2016	VGPR	N/A	01-23-2016
	5	Elo-Rd	02-2016	04-2016	N/A	N/A	04-2016
	6	Dara-Pd	05-2016	09-2016	PR	N/A	N/A
	7	Allo-SCT → Pembro → DLI → Dara-Pd	10-11-2016	08-01-2019	sCR	12-13-2016	04-17-2019
	8	Ipilimumab + Dara-Pd	08-20-2019	10-06-2020	SD	-	10-06-2020
		Leukapheresis	11-24-2020	11-24-2020			
		Bridging therapy:					
		Dara-Pd	12-02-2020	01-02-2021	SD	-	N/A
		MARCH109 infusion	01-22-2021	01-22-2021			
5	1	VRd → Mel200 ASCT → KRd	01-26-2017	01-2019	CR	N/A	01-2019
	2	Dara-Pd	02-22-2019	04-16-2019	SD	-	04-2019
	3	PCd	04-23-2019	05-29-2019	SD	-	06-06-2019
	4	**BCMA-directed CAR T cell therapy**	07-10-2019	07-15-2019	sCR	09-16-2020	11-2019
	5	Dara-Pd	10-07-2020	12-20-2020	SD	-	12-29-2020
		Leukapheresis	01-11-2021	01-11-2021			
		Bridging therapy:					
		Dara-Pd	01-15-2021	01-30-2021	PD	-	02-09-2021

		MARCH109 infusion	02-19-2021	02-19-2021			
6	1	Vd + liposomal doxorubicin →	07-2009	06-03-2010	CR	09-16-2009	03-23-2018
		Mel200 ASCT → R → Allo-SCT		03-27-2018			
	2	VCd	03-27-2018	12-02-2019	SD	-	N/A
	3	KRd	04-05-2018	10-19-2020	sCR	05-21-2018	12-16-2019
	4	Dara-Pd	03-09-2020		PR	05-30-2020	12-14-2020
				01-20-2021			
		Leukapheresis	01-20-2021				
		Bridging therapy:		02-13-2021			
		Dara-Pd	01-25-2021	03-05-2021	PD	-	02-28-2021
		MARCH109 infusion	03-05-2021				
7	1	VCd	01-2013	02-2013	N/A	N/A	N/A
	2	VCd + R	02-2013	04-2013	N/A	N/A	N/A
	3	V-DCEP	04-30-2013	06-2013	N/A	N/A	N/A
	4	Bendamustine-Rituximab	07-16-2013	07-19-2013	N/A	N/A	N/A
	5	Carfilzomib	08-06-2013	08-13-2013	N/A	N/A	N/A
	6	C mobilization → Mel-ASCT → Pd	08-30-2013	2014	N/A	N/A	2014
	7	Daratumumab	04-14-2014	10-06-2015	N/A	N/A	10-06-2015
	8	KRd	11-12-2015	2016	N/A	N/A	2016
	9	Selinexor	N/A	N/A	N/A	N/A	2016
	10	Liposomal doxorubicin + VP	N/A	N/A	N/A	N/A	N/A
	11	Dexamethasone	2017	12-11-2017	SD	-	N/A
	12	Venetoclax-Vd	12-26-2017	01-28-2018	SD	-	N/A
	13	**BCMA-directed CAR T cell therapy**	04-01-2018	04-03-2018	VGPR	07-31-2018	12-17-2019
	14	Venetoclax-Vd	02-08-2020	11-28-2020	SD	-	N/A
		Leukapheresis	01-25-2021	01-25-2021			
		Bridging therapy:					
		Venetoclax-Vd	01-30-2021	02-13-2021	PD	-	03-23-2021
		MARCH109 infusion	04-05-2021	04-05-2021			
9	1	VRd	09-25-2018	12-11-2018	SD	-	-
	2	KCd	12-27-2018	01-10-2019	SD	-	-
	3	VTd → PACE → Mel200 ASCT	02-05-2019	04-08-2019	PR	04-01-2019	06-19-2019
	4	Dara-Pd	07-17-2019	12-28-2020	PR	09-04-2019	01-11-2021

		Leukapheresis	02-10-2021	02-10-2021			
		Bridging therapy:					
		Bendamustine-d	02-12-2021	02-13-2021	SD	-	N/A
		MARCH109 infusion	04-19-2021	04-19-2021			
10	1	KRd	05-13-2016	09-22-2016	sCR	08-24-2016	09-19-2017
	2	Dara-Pd	10-13-2017	01-09-2018	SD	-	-
	3	KRd → Mel140 ASCT → R-Ixa-d	01-19-2018	11-2019	PR	07-20-2018	11-13-2019
	4	Elo-Pd	12-03-2019	02-18-2020	SD	-	03-03-2020
	5	KRd	03-27-2020	04-08-2020	PD	-	04-08-2020
	6	**Belantamab**	04-22-2020	04-22-2020	PD	-	05-06-2020
	7	**BCMA-directed CAR T cell therapy**	05-20-2020	05-25-2020	PD	-	06-10-2020
	8	V-DCEP	06-19-2020	07-25-2020	PR	08-14-2020	N/A
	9	FcRH5-CD3 bispecific antibody	08-26-2020	01-20-2021	PR	11-25-2020	02-03-2021
		Leukapheresis	02-15-2021	02-15-2021			
		Bridging therapy:					
		Bendamustine-Vd	02-17-2021	04-07-2021	SD	-	03-31-2021
		MARCH109 infusion	05-03-2021	05-03-2021			
11	1	VRd	07-14-2015	09-25-2015	PR	08-04-2015	09-15-2015
	2	KRd → R	11-18-2015	08-09-2016	PR	12-01-2015	07-12-2016
	3	MEL200 ASCT → V	09-06-2016	05-01-2018	CR	11-07-2016	05-01-2018
	4	Dara-Vd	05-16-2018	07-24-2018	SD	-	07-24-2018
	5	Dara-Rd	08-07-2018	10-02-2018	SD	-	09-24-2018
	6	KCd	10-09-2018	12-12-2018	PR	10-24-2018	12-11-2018
	7	DCEP	01-17-2019	01-20-2019	PR	01-25-2019	02-08-2019
	8	KCd + T	02-19-2019	02-21-2019	PR	02-25-2019	02-27-2019
	9	**BCMA-directed CAR T cell therapy**	04-11-2019	4-16-2019	sCR	05-14-2019	12-22-2020
	10	Elo-Pd	01-06-2021	01-28-2021	PD	-	02-09-2021
		Leukapheresis	02-17-2021	02-17-2021			
		Bridging therapy:					
		Bendamustine-Vd	02-22-2021	03-25-2021	PD	-	03-25-2021
		Isa-Pd	04-08-2021	04-28-2021	PR	04-28-2021	05-18-2021

		MARCH109 infusion	06-02-2021	06-02-2021			
13	1	KRd + clarithromycin → R	2-11-2015	04-26-2016	N/A	N/A	04-2016
	2	Elo-Rd	05-09-2016	08-23-2016	N/A	N/A	08-2016
	3	K-Mel-d	09-26-2016	05-30-2017	N/A	N/A	05-2017
	4	Nivolumab-Pd	06-20-2017	07-03-2017	N/A	N/A	N/A*
	5	Dara-V-Pd → Mel200 ASCT → V-Pd	04-08-2017	09-18-2018	N/A	N/A	09-2018
	6	Ven-Vd	11-06-2018	01-07-2019	N/A	N/A	N/A
	7	Ven-Kd	01-2019	10-15-2019	N/A	N/A	10-29-2019
	8	Anti-BCMA monoclonal antibody	11-26-2019	12-17-2019	N/A	N/A	12-2019
	9	Selinexor-Kd	12-24-2019	01-07-2020	N/A	N/A	N/A*
	10	Bendamustine-Vd	01-07-2020	03-10-2020	SD	-	03-03-2020
	11	**BCMA-directed CAR T cell therapy**	04-01-2020	04-06-2020	sCR	05-04-2020	12-29-2020
	12	Cyclophosphamide	01-12-2021	01-18-2021	SD	-	N/A
	13	**BCMA-directed CAR T cell therapy**	02-03-2021	02-08-2021	PR	03-09-2021	04-13-2021
		Leukapheresis	04-26-2021	04-26-2021			
		Bridging therapy:					
		Isa-Kd	05-08-2021	05-08-2021	PD	-	06-08-2021
		MARCH109 infusion	06-30-2021	06-30-2021			
14	1	VRd	02-2015	08-2015	N/A	N/A	N/A
	2	KRd → Mel200 ASCT → R	09-03-2015	10-03-2017	VGPR	09-14-2016	09-12-2017
	3	Elo-Rd	10-17-2017	01-09-2018	SD	-	01-09-2018
	4	Dara-Pd	01-23-2018	07-14-2018	SD	-	07-17-2018
	5	**BCMA-directed CAR T cell therapy**	08-30-2018	09-04-2018	sCR	12-11-2018	05-06-2021
		Leukapheresis	05-24-2021	05-24-2021			
		Bridging therapy:					
		None	-	-			
		MARCH109 infusion	07-14-2021	07-14-2021			
15	1	Dara-Krd → Mel200 ASCT → R	06-13-2018	12-2019	sCR	09-19-2018	12-13-2019
	2	Elo-Pd	12-27-2019	01-11-2020	PD	-	01-11-2019
	3	V-DCEP → Dara-Kd	01-16-2020	11-13-2020	sCR	02-24-2020	11-20-2020
	4	Dara-Pd	12-09-2020	12-23-2020	PD	-	12-23-2020
	5	V-DCEP	12-31-2020	01-03-2021	VGPR	02-02-2021	N/A

	6	**BCMA-CD3 bispecific antibody**	02-10-2021	05-14-2021	sCR	03-18-2021	05-12-2021
		Leukapheresis	06-09-2021	06-09-2021			
		Bridging therapy:					
		VCd	07-02-2021	07-02-2021	SD	-	08-27-2021
		MARCH109 infusion	09-13-2021	09-13-2021			
16	1	VCd → Mel200 ASCT → VRd → R	08-18-2014	09-2016	sCR	02-25-2015	08-23-2016
	2	KPd → Pd	09-22-2016	04-2018	VGPR	01-04-2017	N/A
	3	KPd	04-04-2018	10-26-2019	SD	-	10-25-2019
	4	Elo-Pd	11-08-2019	02-01-2020	SD	-	01-31-2020
	5	Dara-Vd	02-08-2020	05-21-2021	VGPR	01-15-2021	06-01-2021
		Leukapheresis	06-14-2021	06-14-2021			
		Bridging therapy:					
		Dara-Vd	06-26-2021	07-23-2021	SD	-	-
		MARCH109 infusion	08-23-2021	08-23-2021			
17	1	KRd	02-2018	08-2018	VGPR	06-2018	09-2018
	2	V-DT-PACE → Mel200 ASCT	10-2018	01-18-2019	PR	03-14-2019	04-2019
	3	Rd	05-2019	09-2019	SD	-	09-2019
	4	Elo-Rd	10-08-2019	11-2019	PD	-	11-2019
	5	Dara-d	11-14-2019	05-20-2020	SD	-	06-02-2020
	6	Bendamustine-Vd → **BCMA-directed CAR T cell therapy**	06-29-2020	07-27-2020	VGPR	12-01-2020	05-25-2021
		Leukapheresis	06-16-2021	06-16-2021			
		Bridging therapy:					
		Isa-Kd	06-17-2021	08-04-2021	PD	-	N/A
		MARCH109 infusion	08-31-2021	08-31-2021			
18	1	VRd → Mel200 ASCT → Allo-SCT → DLI x 3	10-27-2012	05-21-2014	sCR	09-11-2013	04-24-2018
	2	Lenalidomide	05-24-2018	09-04-2018	SD	-	N/A*
	3	Pomalidomide	10-16-2018	01-2019	SD	-	01-30-2019
	4	Dara-Vd	02-12-2019	10-28-2019	SD	-	10-28-2019
	5	KPd + DLI x 1	12-11-2019	10-07-2020	sCR	07-15-2020	04-28-2021

		Leukapheresis Bridging therapy: KPd MARCH109 infusion	07-07-2021 07-14-2021 11-10-2021	07-07-2021 09-21-2021 11-10-2021	 SD	 -	 -
19	1 2 3 4 5 6 7	VRD → Mel200 ASCT → R Vd → V KRd → K DPd VCd Pd **BCMA-directed CAR T cell therapy**	07-15-2010 07-22-2013 01-2015 07-13-2017 05-2018 01-2019 09-24-2019	07-2013 01-2015 07-2017 05-2018 01-2019 07-23-2019 09-24-2019	sCR sCR sCR sCR N/A N/A sCR	08-2010 09-09-2013 06-2015 09-18-2017 N/A N/A 10-21-2019	07-03-2013 01-2015 07-2017 05-2018 01-2019 07-30-2019 06-21-2021
		Leukapheresis Bridging therapy: PCd MARCH109 infusion	07-14-2021 07-2021 09-27-2021	07-14-2021 08-12-2021 09-27-2021	 SD	 -	 09-22-2021

V, bortezomib; C, cyclophosphamide; d, dexamethasone; Mel, melphalan; ASCT, autologous stem cell transplant; R, lenalidomide; K, carfilzomib; P, pomalidomide; Elo, elotuzumab; dara, daratumumab; T, thalidomide; PACE, cisplatin + adriamycin + cyclophosphamide + etoposide combination chemotherapy; BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; Ven, venetoclax; DCEP, dexamethasone + cyclophosphamide + etoposide + cisplatin combination chemotherapy; P, prednisone; Ixa, ixazominb; Allo-SCT, allogeneic stem cell transplant; Pembro, pembrolizumab; DLI, donor lymphocyte infusion; (s)CR, (stringent) complete response; VGPR, very good partial response; partial response, PR; SD, stable disease; progressive disease, PD; N/A not available; * stopped due to toxicity

Table S4: Baseline characteristics of patients with and without delayed cerebellar toxicity

	No delayed toxicity (n=15)	Delayed toxicity (n=2)
Median age, years (range)	60 (38-76)	66 (63-68)
Male, n (%)	12 (80)	1 (50)
High-risk cytogenetics, n (%)*	11 (73)	2 (100)
Extramedullary plasmacytoma, n (%)	7 (47)	0 (0)
BM plasma cells > 30%, n (%)	5 (33)	1 (50)
M-spike >3 gm/dL or FLC >100 mg/dL, n (%)	2 (13)	1 (50)
Prior Lines of Therapy, median (range)	6 (5-14)	8 (5-10)
Refractory to last line, n (%)	14 (93)	2 (100)
Penta-exposed, n (%)	15 (100)	15 (100)
Triple-refractory, n (%)	14 (93)	2 (100)
Prior Autologous Transplant, n (%)	15 (100)	2 (100)
Prior Allogeneic Transplant, n (%)	3 (20)	0 (0)

Prior BCMA therapy, n (%)**	9 (60)	1 (50)
Prior CAR T cell therapy, n (%)	7 (47)	1 (50)
Bridging therapy, n (%)	14 (93)	2 (100)
Refractory to bridging, n (%)	13 (87)	2 (100)

CAR, chimeric antigen receptor; BCMA, B cell maturation antigen.