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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Leahy AB, Newman H, Li Y, et al. CD19-targeted chimeric antigen receptor T-cell therapy for CNS relapsed or refractory acute lymphocytic leukaemia: a post-hoc analysis of pooled data from five clinical trials. *Lancet Haematol* 2021; **8:** e711–22.

Appendix

Supplemental Methods

Outcomes

Data sources:
Patient demographics, baseline characteristics and adverse event (AE) reports for neurotoxicity and cytokine release syndrome (CRS) were obtained from the clinical trial databases. Patient clinical history, including prior radiation treatment, disease status at referral, and neurologic history, was manually abstracted from the medical record, including clinical trial referral and enrollment records. Medications administered following infusion and other post-infusion toxicity management was electronically abstracted from the electronic medical record.

Serious adverse events:
Adverse events were defined as serious when they were life-threatening or resulted in hospitalization or prolongation of hospitalization, congenital anomaly or disability (or required intervention to prevent), or death, or when considered an important medical event by the clinical investigator. Encephalopathy was considered an important medical event and reported as a serious adverse event on these trials.

Supplement to Table 1: *Demographics and baseline clinical characteristics of patients with CD19-positive acute lymphocytic leukaemia or lymphocytic lymphoma by stratum*

The CNS-negative stratum includes 22 patients for whom pre-infusion bone marrow evaluation and lumbar puncture was obtained at enrollment, 3-6 weeks prior to CAR T-cell infusion, and who received bridging chemotherapy in the interim. Disease status at evaluation is as follows: M2 bone marrow, n=4; M3, n=18; CNS1, n=21; CNS2, n=3.

The CNS-positive stratum includes 1 patient for whom whom pre-infusion bone marrow evaluation and lumbar puncture was obtained at enrollment, 3-6 weeks prior to CAR T-cell infusion, and who received bridging chemotherapy in the interim. Disease status at evaluation is as follows: M3 bone marrow, n=1; CNS, n=1.

Appendix Table 1: Inclusion, exclusion, and infusion criteria for patients with CNS disease by clinical trial.

NCT01626495

Initial protocol:
Excluded patients with active CNS involvement with malignancy (i.e. CNS3 for ALL). Patients with prior CNS disease that has been effectively treated will be eligible. Routine CNS prophylaxis for ALL is permitted.

After amendment:
Patients with CNS3 disease will be eligible if CNS disease is responsive to therapy (at infusion, must meet infusion criteria)

Excluded CNS3 disease that is progressive on therapy, or with CNS parenchymal lesions that might increase the risk of CNS toxicity

CNS3 cohort infusion criteria:

1) Disease status:
    a. If CNS3 by spinal fluid involvement, stable/responding disease as indicated by:
        i. stable or decreasing CSF WBC, and
        ii. total CSF WBC < 100 in a sample obtained within 72 hours of CART-19 infusion.
    b. If CNS3 by MRI findings, there must be interval stability or improvement on MRI within 2 weeks of infusion
    c. If CNS3 by cranial nerve findings, there must be stability or improvement of these cranial nerve findings on exam post intervention
2) At least 21 days since any prior CNS3 patient was infused.
3) Patients with CNS3 disease requiring radiation therapy must be at least 8 weeks post radiation at CART-19 infusion
4) No acute/ongoing neurologic toxicity > Grade 1 with the exception of a history of controlled seizures or fixed neurologic deficits that have been stable/improving over the past 3 months

NCT02228096

Excluded active CNS involvement by malignancy, defined as CNS-3 per NCCN guidelines. Note: Patients with history of CNS disease that has been effectively treated will be eligible

NCT02435849

Excluded active CNS involvement by malignancy, defined as CNS-3 per NCCN guidelines. Note: Patients with history of CNS disease that has been effectively treated will be eligible

NCT02374333

Patients with CNS3 disease will be eligible if CNS disease is responsive to therapy (at infusion, must meet infusion criteria)

Excluded CNS3 disease that is progressive on therapy, or with CNS parenchymal lesions that might increase the risk of CNS toxicity

Infusion criteria:

1) For patients with CNS3 disease, 8 weeks from cranial radiation therapy (if used) and stable or improving CNS disease by the following criteria as applicable:
    a. If CNS3 by spinal fluid involvement, CSF WBC count stable or decreasing and <100 by lumbar puncture within 72 hours of infusion
    b. If CNS3 by MRI findings, improvement in MRI findings within 2 weeks of infusion
    c. If CNS3 by cranial nerve findings, stable or improving cranial nerve exam
2) No acute/ongoing neurologic toxicity > Grade 1 with the exception of controlled seizures or fixed neurologic deficits that have been stable/improving over the prior 3 months

NCT02906371

Patients with prior or current history of CNS3 disease will be eligible if CNS disease is responsive to therapy (at infusion, must meet criteria in Section 5.3)

Excluded CNS3 disease that is progressive on therapy, or with CNS parenchymal lesions that might increase the risk of CNS toxicity.

Infusion criteria:

1) Disease status:
    a. If CNS3 by spinal fluid involvement, stable/responding disease as indicated by:
        i. stable or decreasing CSF WBC, and
        ii. total CSF WBC < 100 in a sample obtained within 5 days of CART19 infusion.

b. If CNS3 by MRI findings, there must be interval stability or improvement on MRI within 2 weeks of infusion
c. If CNS3 by cranial nerve findings, there must be stability or improvement of these cranial nerve findings on exam post intervention

2) Patients with CNS3 disease requiring radiation therapy must be at least 8 weeks post cranial radiation at CART19 infusion
3) No acute/ongoing neurologic toxicity > Grade 1 with the exception of a history of controlled seizures or fixed neurologic deficits that have been stable/improving over the past 3 months

Appendix Table 2: Univariate Cox regression for relapse free and overall survival.

	Relapse free survival			*Overall survival*		
	HR	**95% CI**	***p* value**	**HR**	**95% CI**	***p* value**
Age						
3-9·99y	REF	REF	REF	REF	REF	REF
<3y	1·328	0·468-3·768	0·59	1·400	0·494-3·968	0·53
10-17·99y	0·639	0·379-1·076	0·092	0·423	0·241-0·741	0·0027
≥18y	0·629	0·308-1·284	0·20	0·477	0·229-0·996	0·049
Male	REF	REF	REF	REF	REF	REF
Female	1·781	1·114-2·848	0·016	1·728	1·064-2·807	0·027
No history of seizure	REF	REF	REF	REF	REF	REF
Seizure	0·953	0·501-1·814	0·88	1·403	0·751- 2·623	0·29
No history of stroke	REF	REF	REF	REF	REF	REF
Stroke	0·475	0·066-3·417	0·46	1·180	0·289-4·824	0·82
No history of methotrexate toxicity	REF	REF	REF	REF	REF	REF
Methotrexate toxicity	0·720	0·290-1·789	0·48	0·321	0·078-1·311	0·11
No prior HSCT	REF	REF	REF	REF	REF	REF
Prior HSCT	1·053	0·659-1·682	0·83	1·060	0·654-1·718	0·81
No history of brain radiation	REF	REF	REF	REF	REF	REF
Brain radiation	0·548	0·317-0·948	0·031	0·548	0·299-1·007	0·053
No history of total body irradiation	REF	REF	REF	REF	REF	REF
Total body irradiation	1·087	0·682-1·732	0·73	1·141	0·704-1·851	0·59
CNS1 at infusion	REF	REF	REF	REF	REF	REF
CNS2 or 3 at infusion	2·060	1·083-3·920	0·028	2·095	1·068-4·111	0·032
All others	0·665	REF	REF	REF	REF	REF
Isolated CNS		0·380-1·163	0·15	0·496	0·245- 1·001	0·050
CNS-negative stratum	REF	REF	REF	REF	REF	REF
CNSr/r stratum	0·847	0·523-1·370	0·50	0·796	0·471- 1·346	0·40
CTL019	REF	REF	REF	REF	REF	REF
HuCART19	0·659	0·361-1·204	0·18	0·474	0·226-0·993	0·048
Bone marrow disease at infusion						
<0·01%	REF	REF	REF	REF	REF	REF
0·01-4·99%	1·748	0·793-3·852	0·17	2·022	0·798-5·124	0·14
5-25%	1·037	0·354-3·036	0·95	1·989	0·680-5·820	0·21
>25%	4·515	2·606-7·824	<·0001	6·541	3·281-13·037	<·0001

Appendix Table 3: Multivariate Cox regression model for relapse free and overall survival.

	Relapse free survival			*Overall survival*		
	HR	**95% CI**	***p* value**	**HR**	**95% CI**	***p* value**
Age*						
3-9·99y	REF	REF	REF	REF	REF	REF
<3y	1·439	0·468-4·424	0·53	1·860	0·627-5·519	0·26
10-17·99y	0·654	0·386-1·110	0·12	0·496	0·280-0·880	0·016
≥18y	0·456	0·211-0·987	0·046	0·452	0·209-0·976	0·043
Male	REF	REF	REF	REF	REF	REF
Female*	1·145	0·693-1·892	0·60	1·148	0·682-1·933	0·60
No history of brain radiation	REF	REF	REF	REF	REF	REF
Brain radiation*	0·600	0·320-1·123	0·11	0·632	0·318-1·258	0·19
CNS1 at infusion	REF	REF	REF	REF	REF	REF
CNS2 or 3 at infusion*	1·703	0·816-3·556	0·16	1·437	0·681-3·033	0·34
CNS-negative stratum	REF	REF	REF	REF	REF	REF
CNSr/r stratum[§]	1·674	0·912-3·074	0·10	1·806	0·947-3·444	0·073
CTL019	REF	REF	REF	REF	REF	REF
HuCART19*	&	&	&	0·703	0·322-1·535	0·38
Bone marrow disease at infusion*						
<0·01%	REF	REF	REF	REF	REF	REF
M1: 0·01-4·99%	2·141	0·925-4·956	0·076	2·720	1·016-7·281	0·046
M2: 5-25%	1·356	0·432-4·255	0·88	2·571	0·805-8·210	0·11
M3: >25%	5·354	2·778-10·321	<·0001	6·972	3·088-15·742	<·0001

*p<0·1 in univariate analysis

[§]Variable of clinical interest

& p>0·1 in univariate analysis, not included in multivariate model

Appendix Table 4: Post-infusion toxicity medication management.

	CNS-negative (n=129)	CNSr/r (n=66)	*p* value
Received tocilizumab, n(%)	27 (21%)	8 (21%)	0·20
Received steroids	20 (16%)	7 (11%)	0·50
Dexamethasone	4 (3·1%)	2 (3·0%)	>0·9
Hydrocortisone	11 (8·5%)	6 (9·1%)	>0·9
Methylprednisone	11 (8·5%)	4 (6·1%)	0·70
Received anti-epileptic (AE) medication			
Rescue benzodiazepine, n(%)	4 (3·1%)	2 (3·0%)	>0·9
Received treatment dose AE, n(%)	8 (6·2%)	5 (7·6%)	0·80
Levetiracetam (Keppra), n	7	4	§
Phenytoin (Dilantin), n	1	0	§
Lacosamide (Vimpat), n	3	1	§
Phenobarbital, n	1	0	§
Other, n	2	2	§

§ Not applicable

Appendix Table 5: Time to SAE analysis.

		CNS-negative, n = 129		**CNSr/r**, n = 66		***p* value**	
	n	**Time to onset**, d Median (IQR)	**Duration**, d Median (IQR)	**Time to onset**, d Median (IQR)	**Duration**, d Median (IQR)	**Time to onset**	**Duration**
Encephalopathy	39	6 (4.8-9.2)	6 (4-11)	5 (5-6)	4 (3-6)	0·58	0·19
Seizure	15	9 (8.2-12.2)	§	6 (5-11)	§	0·54	§
Movement disorder	1	§	§	18	2	§	§
Speech impairment	1	§	§	8	1	§	§

§ Not applicable

Appendix Table 6: Univariate logistic regression for neurotoxicity.

	Any neurotoxicity			***Grade 3 or 4 neurotoxicity***		
	OR	**95% CI**	***p* value**	**OR**	**95% CI**	***p* value**
Age						
3-9·99y	REF	REF	REF	REF	REF	REF
<3y	0·480	0·107-2·162	0·62	<0·001	<0·001->999·999	0·96
10-17·99y	0·567	0·299-1·074	0·68	0·863	0·323-2·308	0·98
≥18y	0·589	0·256-1·355	0·83	1·250	0·384-4·070	0·96
Male	REF	REF	REF	REF	REF	REF
Female	1·216	0·689-2·145	0·50	0·995	0·414-2·393	0·99
No neurologic comorbidity	REF	REF	REF	REF	REF	REF
Neurologic comorbidity	1·711	0·859-3·411	0·13	2·708	1·080-6·791	0·034
No seizure	REF	REF	REF	REF	REF	REF
Seizure	1·669	0·724-3·846	0·23	1·435	0·447-4·613	0·54
No stroke	REF	REF	REF	REF	REF	REF
Stroke	3·511	0·359-34·363	0·28	2·561	0·255-25·705	0·42
No methotrexate toxicity	REF	REF	REF	REF	REF	REF
Methotrexate toxicity	1·000	0·348-2·874	1·00	1·165	0·246-5·525	0·85
No neurologic deficit	REF	REF	REF	REF	REF	REF
Neurologic deficit	1·908	0·601-6·056	0·27	2·430	0·617-9·576	0·20
No prior HSCT	REF	REF	REF	REF	REF	REF
Prior HSCT	0·789	0·449-1·387	0·41	1·460	0·608-3·511	0·40
No history of brain radiation	REF	REF	REF	REF	REF	REF
Brain radiation	1·732	0·916-3·274	0·090	0·939	0·349-2·524	0·90
No history of total body irradiation	REF	REF	REF	REF	REF	REF
Total body irradiation	0·864	0·489-1·527	0·62	1·552	0·649-3·714	0·32
CNS1 at infusion	REF	REF	REF	REF	REF	REF
CNS2 or 3 at infusion	1·905	0·706-5·142	0·20	0·415	0·053-3·270	0·40
All others	REF	REF	REF	REF	REF	REF
Isolated CNS	1·603	0·810-3·169	0·18	0·495	0·140-1·752	0·28
CNS-negative stratum	REF	REF	REF	REF	REF	REF
CNSr/r stratum	1·946	1·067-3·550	0·030	1·048	0·420-2·616	0·92
CTL019	REF	REF	REF	REF	REF	REF
HuCART19	0.766	0·381-1·538	0·45	0·529	0·149-1·876	0·32
Bone marrow disease at infusion						
<0·01%	REF	REF	REF	REF	REF	REF
0·01-4·99%	0·870	0·356-2·129	0·085	1·690	0·268-10·664	0·78
5-25%	2·393	0·858-6·680	0·22	1·315	0·129-13·399	0·60
>25%	2·581	1·324-5·032	0·030	7·315	2·040-26·228	0·0014

Appendix Table 7: Multivariate logistic regression models for neurotoxicity outcomes: (1) any neurotoxicity, (2) grade 3/4 neurotoxicity.

	OR	95% CI	*p* value
Any neurotoxicity			
No history of brain radiation	REF	REF	REF
Brain radiation*	1·504	0·669-3·382	0·32
CNS1 at infusion	REF	REF	REF
CNS2 or 3 at infusion§	1·266	0·430-3·730	0·67
CNS-negative stratum	REF	REF	REF
CNSr/r stratum*	3·420	1·440-8·121	0·0053
No neurologic comorbidity	REF	REF	REF
Neurologic comorbidity§#	1·572	0·729-3·391	0·25
Bone marrow disease at infusion*			
<0·01%	REF	REF	REF
M1: 0·01-4·99%	1·450	0·529-3·976	0·094
M2: 5-25%	5·458	1·658-17·970	0·063
M3: >25%	5·720	2·415-13·546	0·0033
Grade 3 or 4 neurotoxicity			
CNS1 at infusion	REF	REF	REF
CNS2 or 3 at infusion§	0·195	0·021-1·827	0·15
CNS-negative stratum	REF	REF	REF
CNSr/r stratum§	2·382	0·758-7·488	0·14
No neurologic comorbidity	REF	REF	REF
Neurologic comorbidity*#	4·152	1·430-12·051	0·0088
Bone marrow disease ≤25%	REF	REF	REF
Bone marrow disease >25%*+	11·785	3·603-38·544	<·0001

*p<0·1 in univariate analysis

§Variable of clinical interest

+Categories were combined due to small numbers

#Composite variable combining history of stroke, seizure, methotrexate toxicity and neurologic deficit

Appendix Table 8: Univariate logistic regression model for seizure

	OR	95% CI	*p* value
Age			
3-9·99yo	REF	REF	REF
<3yo	·	·	·
10-17·99yo	0·878	0·293-2·637	0·95
≥18yo	0·290	0·034-2·460	0·97
Male	REF	REF	REF
Female	0·852	0·291-2·495	0·77
No history of seizure	REF	REF	REF
Seizure	2·612	0·765-8·920	0·13
No history of stroke	REF	REF	REF
Stroke	4·214	0·411-43·213	0·23
No history of methotrexate toxicity	REF	REF	REF
Methotrexate toxicity	5·587	1·528-20·433	0·0093
No history of neurologic deficit	REF	REF	REF
Neurologic deficit	4·250	1·031-17·527	0·045
No history of neurologic comorbidity	REF	REF	REF
Neurologic comorbidity	6·682	2·225-20·070	0·0007
No priror HSCT	REF	REF	REF
Prior HSCT	0·935	0·325-2·688	0·90
No history of brain radiation	REF	REF	REF
Brain radiation	1·375	0·447-4·228	0·58
No history of total body irradiation	REF	REF	REF
Total body irradiation	1·197	0·416-3·444	0·11
CNS1 at infusion	REF	REF	REF
CNS2 or 3 at infusion	·	·	·
All others	REF	REF	REF
Isolated CNS	0·522	0·113-2·406	0·40
CNS-negative stratum	REF	REF	REF
CNSr/r stratum	0·975	0·319-2·981	0·97
CTL019	REF	REF	REF
HuCART19	2·000	0·644-6·216	0·23
Bone marrow disease at infusion			
<0·01%	REF	REF	REF
0·01-4·99%	0·604	0·065-5·633	0·29
5-25%	3·282	0·668-16·133	0·13
>25%	1·885	0·527-6·738	0·49

· Sample size too small

Appendix Table 9: Frequency table of association between CRS and any neurotoxicity.

	Grade	No neurotoxicity n = 104	Any neurotoxicity n = 91	Grade 0, 1, or 2 neurotoxicity n = 172	Grade 3 or 4 neurotoxicity n = 23
CRS n (%)	0	25 (24%)	7 (7·7%)	32 (19%)	0
	1	13 (13%)	1 (1·1%)	14 (8·1%)	0
	2	56 (54%)	43 (47%)	97 (56%)	2 (8·7%)
	3	7 (6·7%)	18 (20%)	19 (11%)	6 (26%)
	4	3 (2·9%)	22 (24%)	10 (5·8%)	15 (65%)