Supplementary: Progressive Multifocal Leukoencephalopathy Following Chimeric Antigen Receptor T-Cell Therapy

Supplemental Methods

FAERS

The FDA adverse event reporting system (FAERS) is a global post-marketing surveillance system consisting of voluntary safety reports, as well as mandatory reports by regulations, submitted by healthcare providers, consumers, and manufacturers. For each report, the FAERS database includes administrative information (country, reporter occupation, and reporting year), patient characteristics (age and sex), drug administration (indication, dosage, and route of administration), adverse event (AE) occurrence date, and outcomes (e.g., death).

Duplicated reports in the FAERS may occur in two main ways. First, follow-up reports of the same drug-reaction pair are common and have the same case number with different case versions. Therefore, by linking the case number and version we could select only the latest version of every safety report, as recommended by the FDA. Second, reports of the same event with different case numbers may occur due to various causes, primarily failure in linking follow-up reports to the original and different reporters (e.g., health professionals, manufacturers, and consumers) who report the same event. To identify suspected duplicated reports with different case numbers, we applied an algorithm to screen for reports of the same drug-AE pair with identical values in four key fields: age, sex, event date, and country of occurrence.

The FAERS was screened for reports of axicabtagene-ciloleucel (Kite/Gilead) and tisagenlecleucel (Novartis) between 01/01/2018 and 03/31/2021. The endpoint was PML reporting, coded in the FAERS at the preferred term level of the Medical Dictionary for Regulatory Activities classification. Because the FAERS is a publicly available and anonymized database, institutional review board approval and informed consent were waived.

CIBMTR

The Center for International Blood and Marrow Transplant Research (CIBMTR) registry was queried for PML reports following axicabtagene-ciloleucel and tisagenlecleucel between 01/01/2018 and 12/31/2020. The CIBMTR is a research collaboration between the Medical College of Wisconsin and the National Marrow Donor Program/Be The Match that developed infrastructure for the collection of data on non-HCT cellular therapies. Additionally, the CIBMTR operates the National Cancer Institute-funded Cellular Immunotherapy Data Resource (CIDR), with the objective of collecting, processing, and sharing data on cellular therapies for the treatment of cancer. Cellular therapy data for any CAR T-cell recipient are

collected longitudinally from 190 participating centers and capture approximately 65% of all commercial CAR-T therapy performed in the US (CIBMTR communication). The CIBMTR assures data quality through a multistage error check, on-site data audits, and metrics for on-time data reporting by participating centers. CIBMTR data is collected as part of a data-sharing protocol and informed consent which allows secondary uses of these data. PML was defined by the treating physicians and self-reported.

Statistical analysis

We conducted a disproportionality analysis comparing the proportion of PML events following CAR-T therapy (cases) with the corresponding proportion of other drugs (non-cases), also known as case/non-case analysis. We used the reporting odds ratio (ROR) and the lower bound of the information component 95% credibility interval (IC_{025}) for signal detection. These measures evaluate whether a drug-AE pair is reported higher-than-expected. The 'expected' number is the AE occurrence by any other drug in the entire database. ROR, a frequentist measure, is the pharmacovigilance equivalent of the odds ratio and therefore is more easily interpretable to non-statisticians. IC_{025}, a Bayesian measure that also accounts for disproportionate reporting, has been shown to reduce false positives when a small number of cases is reported.[1–3] A lower bound of the ROR 95% confidence interval greater than one, and a positive IC_{025} value, are the traditional thresholds also used in this study.[4,5]

Continuous normally distributed variables were presented as mean (±standard deviation), while non-normally distributed variables were presented by median [interquartile range (IQR)]. Normality was assessed with the Shapiro–Wilk test and visual inspection of quantile-quantile plots and histograms. Between-group comparisons were performed by the Mann-Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical ones, respectively. Incidence was calculated by the number of new PML cases divided by the total number of CD19-CAR-T recipients, with a 95% CI measured by the binomial distribution. All tests were two-sided with significance levels defined as P-values <0.05. Data processing and statistical analysis were performed in R statistical software version 3.6.0 (R Foundation for Statistical Computing).

References

1. Bate A, Lindquist M, Edwards IR, et al. A Bayesian neural network method for

adverse drug reaction signal generation. *Eur J Clin Pharmacol*. 1998;54(4):315-321. doi:10.1007/s002280050466

2. Bate A, Evans SJW. Quantitative signal detection using spontaneous ADR reporting. *Pharmacoepidemiol Drug Saf*. 2009;18(6):427-436. doi:10.1002/pds.1742

3. Norén GN, Hopstadius J, Bate A. Shrinkage observed-to-expected ratios for robust and transparent large-scale pattern discovery. *Stat Methods Med Res*. 2013;22(1):57-69. doi:10.1177/0962280211403604

4. Harpaz R, Dumouchel W, Lependu P, Bauer-Mehren A, Ryan P, Shah NH. Performance of pharmacovigilance signal-detection algorithms for the FDA adverse event reporting system. *Clin Pharmacol Ther*. 2013;93(6):539-546. doi:10.1038/clpt.2013.24

5. Harpaz R, Dumouchel W, Shah NH, Madigan D, Ryan P, Friedman C. Novel data-mining methodologies for adverse drug event discovery and analysis. *Clin Pharmacol Ther*. 2012;91(6):1010-1021. doi:10.1038/clpt.2012.50

Supplemental Table 1: Demographic and clinical details of CAR-T-associated PML reports in the FAERS.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11
Age (yrs), Sex, Weight.	58, M, 88.5 kg	11, M.	65, F, 115kg	62, F, 76 kg.	56, F, 53 kg	76, F.	64, F, 64 kg	68, F	76, F, 48.6 kg	NA	58, M, 49 kg
Country	Netherland	U.S.	U.S.	U.K.	Netherlands	U.S.	Switzerland	U.S.	U.S.	U.K.	Japan
Reporter	Physician	Consumer	Physician	Health-professional	Health-professional	Health-professional	Physician	Physician	Physician	Physician	Physician
CAR-T type (treatment date)	Axi-cell (18-Jul-2019)	Tisagenlecleucel	Axi-cell (07-Jan-2019)	Axi-cell	Tisagenlecleucel (07-May-2020)	Axi-cell (2018)	Axi-cell (17-March-2020)	Axi-cell	Axi-cell (29-October-2018).	tisagenlecleucel	Tisagenlecleucel (18-Dec-2019)
Indication (diagnosis date)	DLBCL (10-July-2018).	ALL	Follicular Lymphoma (17-Nov-2015)	NHL	Lymphoma	DLBCL	DLBCL	DLBCL	NA	Lymphoma	DLBCL (05-Nov-2018)
Disease status	NA	Active disease at time of PML diagnosis.	Active disease at time of PML diagnosis.	Active disease at time of PML diagnosis.	NA	Complete response to CAR-T. Remission at time of PML diagnosis.	NA	Complete response to CAR-T. Remission at time of PML diagnosis.	Complete response to CAR-T. Remission at time of PML diagnosis.	Active disease at time of PML diagnosis.	Complete response to CAR-T. Unclear disease status at time of PML diagnosis.
Prior treatments	▪ R-CHOP ▪ Flu+Cyclo	▪ Chemotherapy. ▪ steroids. ▪ Radiotherapy. ▪ Allo-HSCT ▪ Teculizumab (TMA)	▪ Bendamustine + RIT. ▪ R-CHOP ▪ Flu+Cyclo	▪ R-CHOEP ▪ R-ICE ▪ R-benda ▪ R-DHAP	NA	▪ R-CHOP ▪ Methotrexate. ▪ Radiotherapy. ▪ R-EPOCH ▪ Auto-HSCT. ▪ Flu+Cyclo	NA	▪ R-CHOP. ▪ R-ICE. ▪ R-BEAM. ▪ auto-HSCT. ▪ Rituximab, Gemcitabine, Oxaliplatin ▪ Flu+Cyclo	▪ Intrathecal Methotrexate	NA	▪ R-CHOP ▪ R-DA-EPOCH ▪ DEVIC ▪ Flu+Cyclo
Concomitant medications	Cotrimoxazole, Valacyclovir, Pantoprazole, Metoclopramide, Levetiracetam, Meropenem, Ondansetron.	NA	Ativan, Synthroid, Allopurinol, Atrovastatin, Coumadin, SMP-TMX.	Mirtazapine, IVIG.	Duloxetine, Omeprazole, Tramadol, Levetiracetam, Folic acid, Pentamidine, Filgrastim.	Acyclovir, TMP-SMX.	Aspirin, Pantoprazole, Bilol, Zolpidem.	Tocilizumab (CRS), Steroids (ICANS).	NA	Acyclovir	IVIG, Antiepileptics (unspecified), Tocilizumab (CRS).
Comorbidities	NA	NA	HF, Anxiety, Hypercholesterolemia, PE,	NA	Depression, Neuropathy,	NA	Sleep disorder, Hypertension.	NA	Neuropathy	NA	Alcohol, Smoking.

			Gout, Hypothyroidism, Carpal tunnel, Cholecystectomy		Easy bruising, Hypogammaglobulinemia, Pancytopenia.						
PML diagnosis	MRI- bilateral frontal white matter abnor. CSF- positive JCV.	CT- changes suggestive of PML	MRI- Abnor. enhancement in the left occipital lobe. Biopsy- confirmed PML.	MRI- typical subcortical white matter hyper- & hypo Intensities. CSF- negative JCV.	MRI- Multifocal white matter abnor. CSF- Unknown.	MRI- subcortical supratentorial white matter disease with a left paramedian pontine hyperintensity (progressed on repeated MRI). CSF- positive JCV.	MRI- Intra-cranial lesion. CSF- positive JCV. Biopsy- confirmed PML.	MRI- bilateral enhancing lesions in the white matter. PET-CT- no FDG-avid lesions. Blood- PCR for JCV detected 5800 DNA copies/ml. CSF- initially negative JCV, repeated test positive. Biopsy- confirmed PML.	MRI- diffuse white matter disease. CSF- positive JCV.	NA	NA
Time from CAR-T inf. To PML (months)	6	31	21	8	8	12	8	7	16	NA	8
Clinical features	Confusion, amnesia, right hand weakness.	Depressed consciousness level.	NA	Distal sensory loss, bilateral leg weakness, poor coordination.	Aphasia, bradyphrenia, ptosis, fine motor disorder.	NA	Blurred vision.	Confusion, aphasia, ataxia, involuntary movements, amnesia, cognitive decline.	Dysarthria, vertigo, ataxia, impaired taste.	NA	Incoherent behavior.
Labs	NA	NA	NA	CD4 count 173 cells/ml.	NA	Immunoglobulin G 255 mg/dl	NA	CD4 count 199 cells/ml	NA	NA	NA
Other post-CAR-T complications	ICANS, Femur fracture, Pneumonia.	NA	AKI.	NA	ICANS, Pancytopenia.	Prolonged pancytopenia, Hypogammaglobulinemia.	NA	Hypogammaglobulinemia, CRS GII ICANS GII, Prolonged neutropenia	NA	Cytopenia, B-cell aplasia, Infections.	CRS GI, Prolonged cytopenia, Neutropenia.
Outcome	Death	death	Death	Death	Unknown	Death	Alive (at last follow-up)	Alive (12 months).	Death	NA	Death
Notes	NA	JCV infection mentioned but	NA	Patient declined brain biopsy.	NA	PET-CT- complete remission one	PML was treated with	PML was treated with mefloquine,	NA	Poor data documentation.	NA

		details not given				month after CAR-T.	Pembrolizumab.	IVIG, Mirtazapine with partial improvement.			

Abbreviations: FAERS - FDA adverse event reporting system; ALL-Acute lymphocytic leukemia; Axi-cell- Axicabtagene Ciloleucel; CRS- cytokine release syndrome; DLBCL- Diffuse large B-cell lymphoma; ICANS- Immune effector cell-associated neurotoxicity syndrome; NA- not available; NHL- Non-Hodgkin lymphoma; U.K.- United Kingdom; U.S.- United States.

Supplemental Table 2: Demographic and clinical details of CAR-T-associated PML cases in the center for international blood and marrow transplant research (CIBMTR) registry.

No.	Event year[a]	Age (years)	Sex	Country	Product	Indication	PML report	Time to onset[b] (months)	Outcome
1	2019	70	Female	USA	Axicabtagene ciloleucel	NHL	Definite	7.5	Alive
2	2020	12	Male	USA	Tisagenlecleucel	ALL	Likely	8.9	Alive
3	2020	75	Female	USA	Axicabtagene ciloleucel	NHL	Definite	16.0	Dead

The CIBMTR registry included 3,328 patients who received axicabtagene-ciloleucel or tisagenlecleucel between 2018 and 2020, among whom 4 have developed PML after the treatment.

a. Year of PML occurrence.
b. Time from treatment initiation to PML onset in months.

Abbreviations: ALL- Acute lymphoblastic leukemia CAR-T- Chimeric antigen receptor T-cell therapy, JCV- John Cunningham virus, NHL- Non-Hodgkin lymphoma, PML- progressive multifocal leukoencephalopathy.