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Human Herpesvirus-6 Reactivation and Disease Are Infrequent in Chimeric Antigen Receptor T-cell Therapy Recipients

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Abstract:
Human herpesvirus-6B (HHV-6B) reactivation and disease are increasingly reported after CAR-T-cell therapy (CARTx). HHV-6 reactivation in the CAR-T-cell product was recently reported, raising questions about product and patient management. Due to overlapping manifestations with immune effector cell-associated neurotoxicity syndrome, diagnosing HHV-6B encephalitis is challenging. We provide two lines of evidence assessing the incidence and outcomes of HHV-6B after CARTx. First, in a prospective study with weekly HHV-6B testing for up to 12 weeks post-infusion, HHV-6B reactivation occurred in eight of 89 participants; three had chromosomally integrated HHV-6 and were excluded, resulting in a cumulative incidence of HHV-6B reactivation of 6% (95% confidence interval (CI), 2.2-12.5%). HHV-6B detection was low level (median peak, 435 copies/mL; IQR, 164-979) and did not require therapy. Second, we retrospectively analyzed HHV-6B detection in blood and/or cerebrospinal fluid (CSF) within 12 weeks post-infusion in CARTx recipients. Of 626 patients, 24 had symptom-driven plasma testing with detection in one. Among 34 patients with CSF HHV-6 testing, one patient had possible HHV-6 encephalitis for a cumulative incidence of 0.17% (95% CI, 0.02-0.94%), although symptoms improved without treatment. Our data demonstrate that HHV-6B reactivation and disease are infrequent after CARTx. Routine HHV-6 monitoring is not warranted. -

Conflict of interest: COI declared - see note

COI notes: Disclosure of Conflicts of Interest A.J.C. has served as consultant and participated in the advisory board, or steering committee for Janssen, BMS, Sebia, Sanofi, Adaptive Biotechnologies; has received research funding from Abbvie, Sanofi, Janssen, BMS, Adaptive Biotechnologies, Nektar, Harpoon, Regeneron, Caelum, IGM Biosciences. D.J.G. has served as an advisor and has received research funding and royalties from Juno Therapeutics, a Bristol-Myers Squibb company; has served as an advisor and received research funding from Seattle Genetics; has served as an advisor for GlaxoSmithKline, Celgene, Ensoma, Janssen Biotech, and Legend Biotech; and has received research funding from SpringWorks Therapeutics, Sanofi, and Cellectar Biosciences. J.G. has served as ad hoc consultant and has received honoraria from Sobi, Legend Biotech, Janssen, Kite Pharma, MorphoSys; research funding from Sobi, Juno Therapeutics (a Bristol-Myers Squibb company), Celgene (a Bristol-Myers Squibb company), Angiocrine Bioscience; and has participated in the independent data review committee for Century Therapeutics. D.M.Z. has received research funding from Merck and has served as a consultant for Allovir for service on endpoint adjudication committees for two trials. M.J.B has served as consultant and has received research funding from Merck; has served as consultant for Symbio, Helocyte, Moderna and Allovir; has served as consultant and had option to acquire stock for EvrysBio. J.A.H. has served as a consultant for Moderna, Allovir, Gilead, SentiBio, Modulus, and Allogene and received research funding from Allovir, Gilead, and Merck. All other authors do not report any conflicts of interest.

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Brief Report

Human Herpesvirus-6 Reactivation and Disease Are Infrequent in Chimeric Antigen Receptor T-cell Therapy Recipients

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DATA AVAILABILITY

The datasets generated and analyzed for this study are available from the corresponding author after publication upon reasonable request, with investigator financial support, and with appropriate documentation of IRB approval and/or data access agreements as applicable.

Key Points

- HHV-6 reactivation in plasma occurred in 6% and possible HHV-6 encephalitis in 0.2% of patients within 12 weeks after CAR-T-cell therapy.
- HHV-6 reactivation and disease are infrequent after CAR-T-cell therapy. Routine HHV-6 monitoring is not warranted.

Explanation of Novelty

This is the first study to systematically evaluate HHV-6B reactivation and disease in CAR-T-cell therapy recipients, including both CD19- and BCMA-targeted CAR-T-cell therapies. Further, we used innovative sampling methods for home-based self-collection of blood to support robust monitoring for up to 12 weeks after CAR-T-cell infusion, establishing the feasibility of this emerging strategy for improved monitoring in cellular therapy recipients.

Abstract

Human herpesvirus-6B (HHV-6B) reactivation and disease are increasingly reported after CAR-T-cell therapy (CARTx). HHV-6 reactivation in the CAR-T-cell product was recently reported, raising questions about product and patient management. Due to overlapping manifestations with immune effector cell-associated neurotoxicity syndrome, diagnosing HHV-6B encephalitis is challenging. We provide two lines of evidence assessing the incidence and outcomes of HHV-6B after CARTx. First, in a prospective study with weekly HHV-6B testing for up to 12 weeks post-infusion, HHV-6B reactivation occurred in eight of 89 participants; three had chromosomally integrated HHV-6 and were excluded, resulting in a cumulative incidence of HHV-6B reactivation of 6% (95% confidence interval (CI), 2.2–12.5%). HHV-6B detection was low level (median peak, 435 copies/mL; IQR, 164–979) and did not require therapy. Second, we retrospectively analyzed HHV-6B detection in blood and/or cerebrospinal fluid (CSF) within 12 weeks post-infusion in CARTx recipients. Of 626 patients, 24 had symptom-driven plasma testing with detection in one. Among 34 patients with CSF HHV-6 testing, one patient had possible HHV-6 encephalitis for a cumulative incidence of 0.17% (95% CI, 0.02–0.94%), although symptoms improved without treatment. Our data demonstrate that HHV-6B reactivation and disease are infrequent after CARTx. Routine HHV-6 monitoring is not warranted.

Introduction

Reactivation of latent viral infections causes substantial morbidity in immunocompromised patients.[1] Human herpesvirus-6B (HHV-6B) is well-established as the most frequent infectious cause of encephalitis after allogeneic hematopoietic cell transplant (HCT), resulting in high mortality and frequent long-term sequelae.[2] Additionally, HHV-6B detection in the blood after allogeneic HCT is associated with delirium and neurocognitive decline.[3] The epidemiology and clinical significance of HHV-6B reactivation is not well elucidated after CAR-T-cell therapy (CARTx). Cases of HHV-6B encephalitis after CARTx have been reported but estimates of the incidence are unknown.[4–9] Overlapping clinical manifestations between viral encephalitis and immune effector cell-associated neurotoxicity syndrome (ICANS)[5] makes diagnosis challenging without systematic testing. Importantly, the frequent occurrence of ICANS in 40–77% of CARTx recipients[10] raises suspicion for a link with HHV-6B reactivation and associated neurologic dysfunction in this population.[3]

Recently, the potential role of cellular therapies as a source of viral infection in immunocompromised recipients was suggested.[11] Using single-cell sequencing, Lareau et al identified a rare population of cells with high HHV-6B transcriptional activity in CD4+ T-cells from in vitro cultures of pre-infusion CAR-T-cells in addition to in vivo cultures from post-infusion patient blood. These data have important implications for manufacturing and screening of T-cell therapeutics, as well as monitoring of patients. Considering these results and given the potential impact of this ubiquitous lymphotropic and neurotropic virus on ICANS, there is renewed interest to explore the kinetics and clinical presentation of HHV-6B after CARTx. We herein provide two lines of compelling clinical evidence demonstrating infrequent detection of HHV-6 DNA in plasma and cerebrospinal fluid (CSF) after CARTx, with no temporal association with ICANS.

Methods

First, we conducted a prospective study to assess the incidence, kinetics of and risk factors for HHV-6B reactivation in adults receiving CARTx for hematologic malignancies from August 2021 through March 2023 at Fred Hutchinson Cancer Center (FHCC). We obtained plasma once pre-lymphodepleting chemotherapy and weekly post-CARTx for up to 12 weeks. For samples after week four, participants had the option to use a novel device (Tasso, Inc; Seattle, WA) for home-based blood self-collection and shipment to our center (**Supplement**). We tested for HHV-6 using PCR;[12] HHV-6 species and inherited chromosomally integrated HHV-6 (iciHHV-6) were determined by droplet digital PCR.[13] Inherited ciHHV-6 occurs when HHV-6 integrates into the chromosome of a germ cell that is subsequently fertilized, resulting in a copy of the HHV-6 genome in every nucleated cell. As a result, all cellular samples (or samples contaminated by cell lysis, e.g., plasma), will have detection of HHV-6 DNA that is not indicative of viral replication (**Supplement**).[14] HHV-6 detection was defined as one or more results >50 copies/mL, the lower limit of detection of the assay.[12] Next, to estimate the incidence of HHV-6 encephalitis after CARTx, we retrospectively analyzed clinical test results for HHV-6B in blood and/or CSF within 12 weeks post-CARTx in adults receiving CARTx for hematologic malignancies at FHCC from July 2013 to January 2023. The studies were approved by the FHCC Institutional Review Board, and all participants provided written informed consent.

Cytokine release syndrome (CRS) and ICANS were graded according to the American Society for Transplantation and Cellular Therapy Consensus Guidelines[15] (or CTCAE criteria v4.03 for CARTx prior to 2019[16]). We calculated the cumulative incidence of HHV-6B reactivation and encephalitis within 12 weeks after CARTx treating death, subsequent CARTx or HCT as competing risks. We stratified cumulative incidence curves by pre-defined baseline variables and used Gray's test to compare between groups. Analyses were performed using Stata/SE (version 17.0, Stata Corp LLC) and SAS version (9.4 TS1M3) (SAS Institute).

The studies were approved by the Fred Hutch Institutional Review Board, and all participants provided written informed consent.

Results and Discussion

HHV-6 reactivation is infrequent after CARTx

In the prospective study, we enrolled 84 participants receiving 89 CAR-T-cell infusions (**Table S1**). HHV-6 viremia occurred in eight participants, three of whom (38%) had iciHHV-6 and were excluded from analyses, resulting in a cumulative incidence of HHV-6 (all species B) reactivation of 6% (95% confidence interval (CI), 2.2–12.5%) within 12 weeks after CARTx (**Figure 1A-1B**). All HHV-6B detection occurred 2–6 weeks post-infusion (median, 21 days). Four of five participants had a single positive result; the remaining participant had HHV-6B detection at weeks 2-4 but no subsequent testing. The median peak viral load was 435 copies/mL (IQR, 164–979).

The incidence of HHV-6B reactivation was similar after CD19/CD20 versus BCMA-targeted CARTx (**Figure 1C**). All participants with HHV-6B reactivation had preceding cytokine release syndrome (CRS), and three had preceding or concomitant ICANS (**Figure 1D, Table S1**). HHV-6B reactivation occurred after ICANS onset in two participants, and the additional participant had a single positive test on the day of symptoms onset attributed to ICANS. Clinical courses are detailed in **Figure 2** and the **Supplement**. No patients had evidence of HHV-6B encephalitis, and none received antiviral therapy active against HHV-6B. Among the three individuals with iciHHV-6, none were diagnosed with ICANS (**Supplement**).

HHV-6 encephalitis is rare after CARTx

We retrospectively analyzed HHV-6B detection in blood and/or CSF testing within 12 weeks post-CARTx in 626 adult CARTx recipients (**Table S2**). Overall, 255 of 626 patients (41%) had ICANS of any grade. Twenty-four patients (3.8% overall), including 19 (7.5%) of 255 patients with ICANS, had plasma HHV-6B testing with detection in one. Altered mental status was the main indication for testing (50%; **Table S3**). Thirty-four patients (5.4% overall), including 30 (11.8%) of 255 patients with ICANS, had CSF tested with PCR for HHV-6B and other viruses in the context of neurologic symptoms (**Table S4**). Cerebrospinal fluid testing practices evolved over time: testing was performed more frequently for neurologic symptoms before 2018; after 2018 testing was limited to patients with ICANS grade ≥3 or atypical symptoms (**Figure S1**).

Cerebrospinal fluid testing detected HHV-6B in one patient 32 days post-infusion with a viral load of 1,100 copies/mL. This was also the one patient with HHV-6B detection in the plasma, and they had two consecutive positive tests with a peak viral load of 1,900 copies/mL. The patient was diagnosed with ICANS grade 4; HHV-6B encephalitis was considered but not treated given the absence of typical features of HHV-6B encephalitis and improvement with corticosteroids. Based on these data, the estimated cumulative incidence of HHV-6B encephalitis was 0.17% (95% CI, 0.02–0.94%) including this possible case. Empiric treatment for HHV-6B in the absence of positive blood and/or CSF detection was not administered per standard practice at Fred Hutch.

Our study provides two lines of compelling clinical evidence demonstrating infrequent detection of HHV-6B DNA in plasma and CSF after CARTx, with no evident temporal association with ICANS. HHV-6B viremia was infrequent, low level, and transient without clear sequelae after CARTx. Furthermore, 38% of participants with HHV-6B detection on systematic screening had iciHHV-6, indicating that routine testing would be almost as likely to incidentally identify latent iciHHV-6, potentially leading to unnecessary treatment.[17] Published guidelines do not

recommend monitoring or preemptive therapy for HHV-6B after allogeneic HCT, even in the highest risk groups such as cord blood HCT recipients, in whom reactivation occurs in up to 90% and HHV-6B encephalitis in up to 10%.[14,18] Although antivirals with activity against HHV-6B are available, they have toxicities that warrant careful stewardship, and preemptive strategies do not appear to prevent encephalitis.[19–23] Thus, routine testing of CAR-T-cell products and post-infusion monitoring for HHV-6B are not supported by clinical data.

The study by Lareau et al provides first evidence of reactivation of latent viruses in CAR-T-cell products, although reactivation of HHV-6 and other viruses in cell cultures has been previously demonstrated.[24] Taken alone, these data raise concern for viral transmission to vulnerable patients via CAR-T-cell products and have potentially important implications for product manufacturing, regulatory requirements, and monitoring of treated individuals. In the patient samples studied by Lareau et al, it is not possible to determine whether the source of HHV-6B was the CAR-T cells or endogenous cells,[25] but our data suggest that the clinical significance is limited.

To the authors' knowledge, this is the first study to systematically evaluate HHV-6B reactivation and disease in CARTx recipients including both CD19- and BCMA-targeted CARTx. Innovative sampling methods for home-based self-collection of blood were used to support robust monitoring for up to 12 weeks after CARTx, establishing the feasibility of this emerging strategy in cellular therapy recipients. The retrospective assessment of HHV-6B detection in blood and CSF in a large cohort offers further insights into the infrequent detection of HHV-6 and related complications in clinical practice. Study limitations include the single-center design and the lack of CSF testing in the large majority of patients with neurological symptoms precluding any estimate of the true incidence of HHV-6B encephalitis. Larger studies may reveal associations between HHV-6B reactivation and other outcomes after CARTx.[14] No patients in our study received allogeneic CAR-T-cell therapy, which may confer increased risk due to more intensive

immunosuppression, highlighting the need to remain diligent in assessing infectious complications of novel therapies.

Conclusion

Our findings suggest that HHV-6B reactivation and disease are infrequent after CARTx. Although routine monitoring is not supported by our findings, HHV-6B testing should be performed in patients with refractory or atypical neurologic symptoms.

DATA AVAILABILITY

The datasets generated and analyzed for this study are available from the corresponding author after publication upon reasonable request, with investigator financial support, and with appropriate documentation of IRB approval and/or data access agreements as applicable.

Acknowledgements

We would like to thank Alythia Vo, Winnie L. Liu and Clementine Chalal (Fred Hutchinson Cancer Center) for data collection; Haiying Zhu and Tracy Santo (University of Washington) for performing laboratory testing of samples; Ryan S. Basom and Chris Davis (Fred Hutchinson Cancer Center) for support with data extraction; Cameron Turtle, Mazyar Shadman, Brian Till, Ryan D. Cassaday, Aude Chapuis and David G. Maloney (Fred Hutchinson Cancer Center) for expert input.

Author Contributions

E.K. and J.A.H. and were responsible for the design of the study and interpretation of the data. E.K., J.A.H., E.M.K., and H.X., analyzed the data and created the figures. E.K., S.S.I., E.S.K, M.K.S., and J.A.H. enrolled participants, collected samples and data. E.C.L., A.J.C., A.P., D.J.G., A.A., J.J.H., J.G collected data. A.C.P.-O. and K.R.J. supervised the laboratory work. E.K. and J.A.H. prepared the first draft of the manuscript. All authors contributed to the writing and revision of the manuscript and approved the final version.

Disclosure of Conflicts of InterestA.J.C. has served as consultant and participated in the advisory board, or steering committee for Janssen, BMS, Sebia, Sanofi, Adaptive

Biotechnologies; has received research funding from Abbvie, Sanofi, Janssen, BMS, Adaptive Biotechnologies, Nektar, Harpoon, Regeneron, Caelum, IGM Biosciences.

D.J.G. has served as an advisor and has received research funding and royalties from Juno Therapeutics, a Bristol-Myers Squibb company; has served as an advisor and received research funding from Seattle Genetics; has served as an advisor for GlaxoSmithKline, Celgene, Ensoma, Janssen Biotech, and Legend Biotech; and has received research funding from SpringWorks Therapeutics, Sanofi, and Cellectar Biosciences.

J.G. has served as ad hoc consultant and has received honoraria from Sobi, Legend Biotech, Janssen, Kite Pharma, MorphoSys; research funding from Sobi, Juno Therapeutics (a Bristol-Myers Squibb company), Celgene (a Bristol-Myers Squibb company), Angiocrine Bioscience; and has participated in the independent data review committee for Century Therapeutics.

D.M.Z. has received research funding from Merck and has served as a consultant for Allovir for service on endpoint adjudication committees for two trials.

M.J.B has served as consultant and has received research funding from Merck; has served as consultant for Symbio, Helocyte, Moderna and Allovir; has served as consultant and had option to acquire stock for EvrysBio.

J.A.H. has served as a consultant for Moderna, Allovir, Gilead, SentiBio, Modulus, and Allogene and received research funding from Allovir, Gilead, and Merck.

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FIGURES

Figures in the Visual Abstract created with BioRender.com

Figure 1

(title) Cumulative incidence and kinetics of HHV-6B detection within 12 weeks after CAR-T-cell infusion

(legend)

A. Heatmap of HHV-6B reactivation kinetics post-CAR-T-cell therapy. Three participants with inherited chromosomally integrated HHV-6 (iciHHV-6) had HHV-6 detection at every tested time point and were excluded from this plot. Each row represents a patient and each square a plasma sample. The intensity of color represents the viral load (negative samples are depicted as white). BCMA-CARTx recipients are depicted at the bottom and CD19/CD20 CARTx recipients at the top of the heat map. **B.** Cumulative incidence curve of HHV-6B reactivation within 12 weeks post-CAR-T-cell therapy: 6% (95% confidence interval (CI), 2.2%–12.5%). **C**. Cumulative incidence of HHV-6B reactivation stratified by CAR-T-cell target: BCMA, 6% (95% CI, 0.4–24.2%) versus CD19/20, 6% (95% CI, 1.9–13.5%). **D.** Cumulative incidence of HHV-6B reactivation stratified by prior CRS and/or ICANS, starting at week 2 after CAR-T-cell infusion as a landmark analysis: prior CRS and/or ICANS, 7.9% (95% CI, 2.9–16.3%) versus no prior CRS and/or ICANS, 0%.

In all curves, death was treated as a competing risk. Gray's test was used for statistical comparisons.

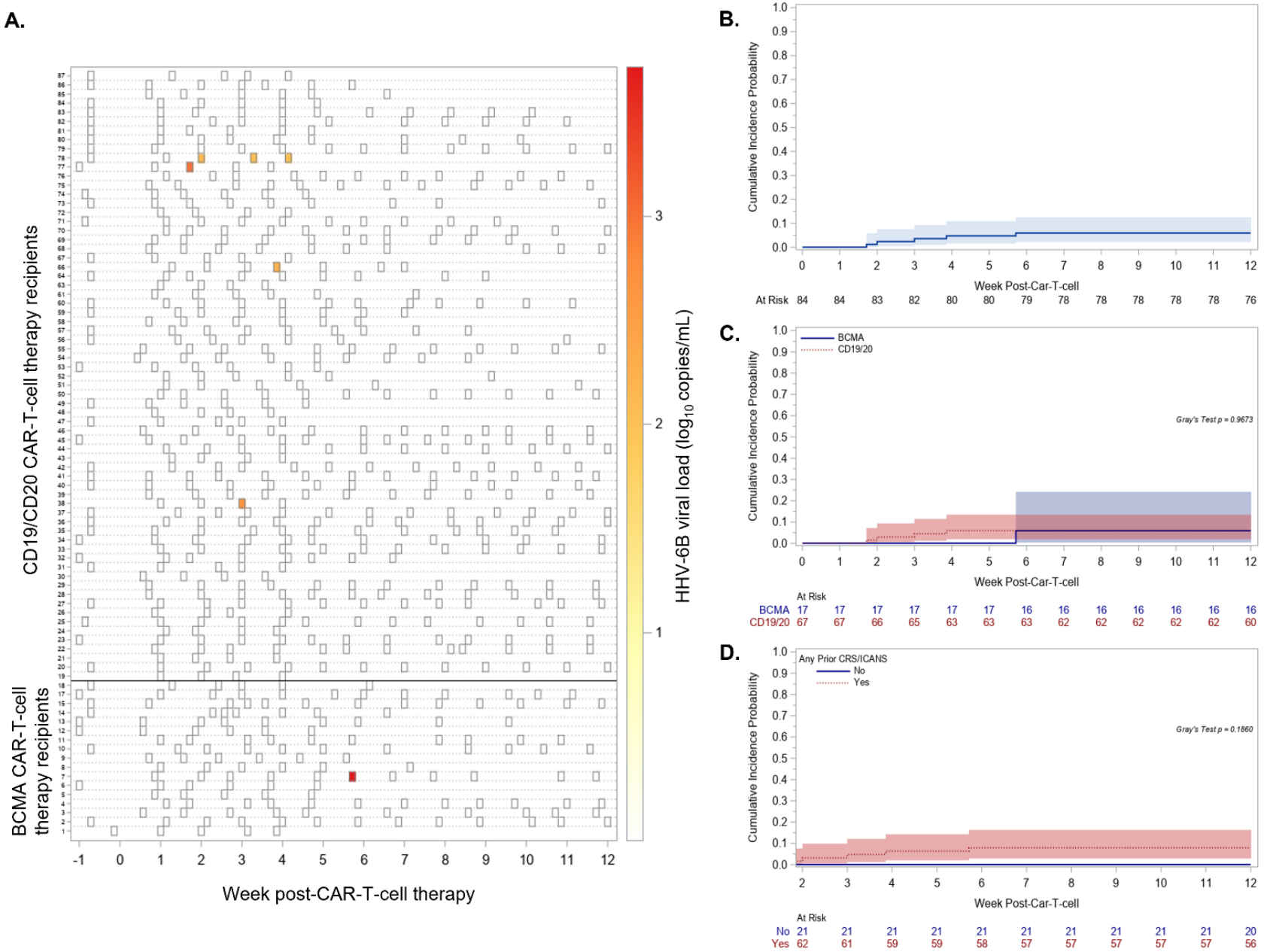

Figure 2

(title) Clinical course of participants with HHV-6 reactivation after CAR-T-cell therapy

(legend) Swimmer plot of clinical courses of participants with HHV-6 reactivation depicting clinical events (CRS, ICANS), management, HHV-6 detection in plasma, and outcomes. Each row depicts an individual participant.

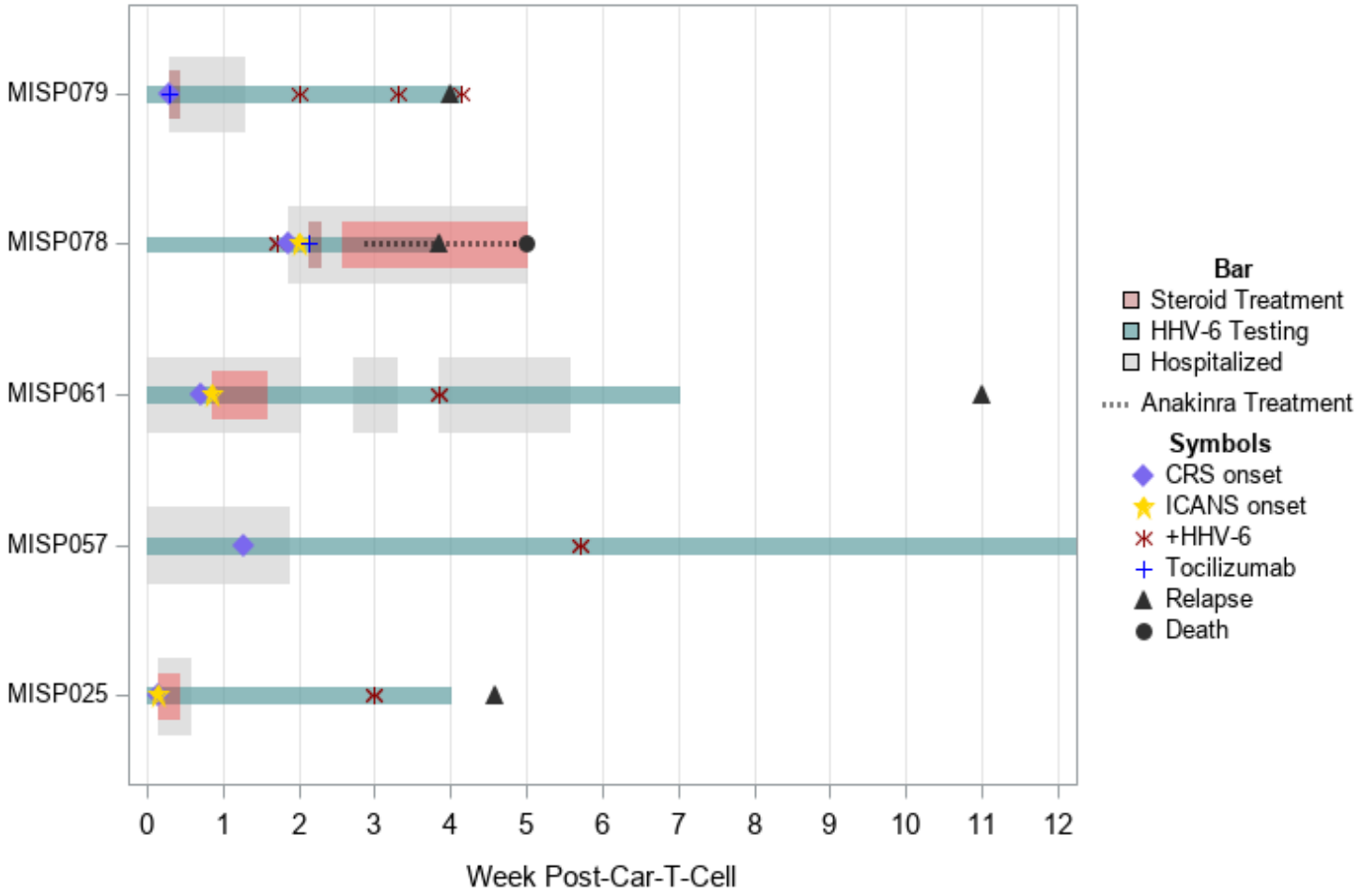

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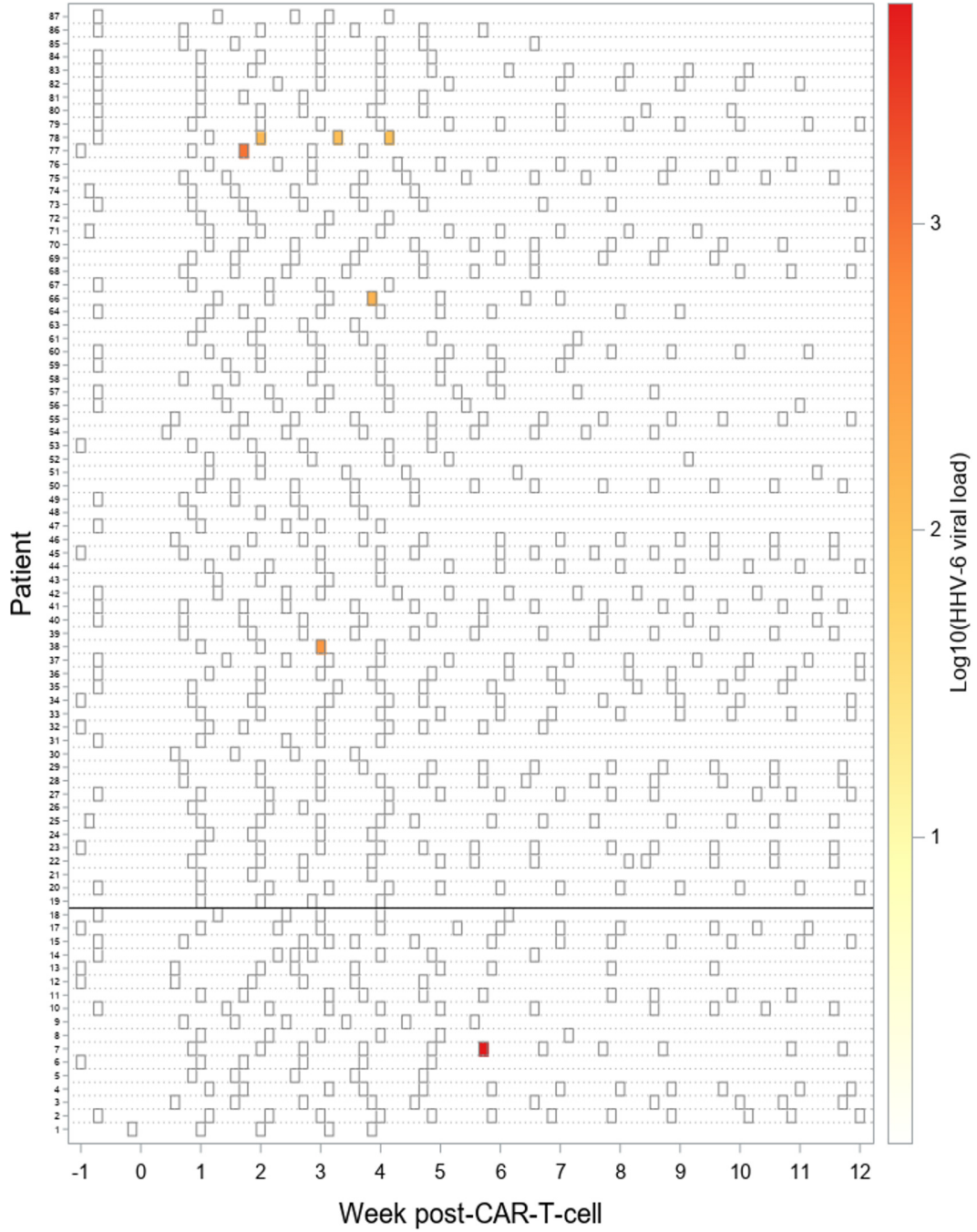
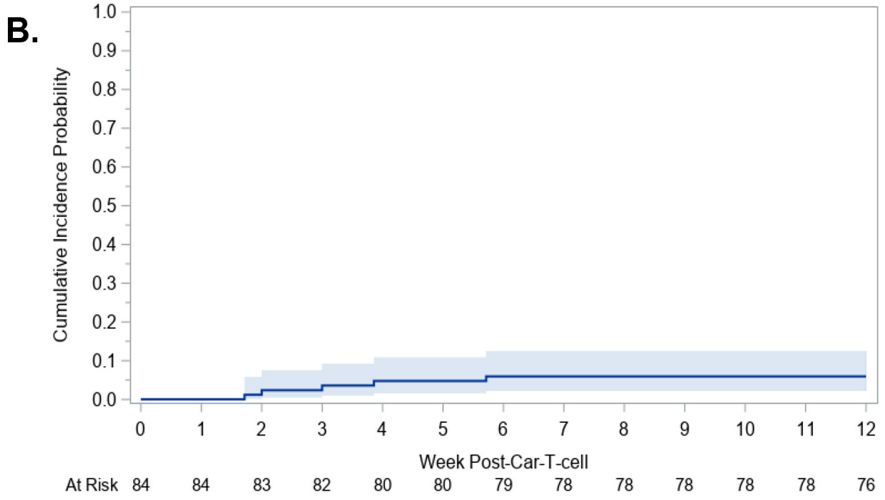
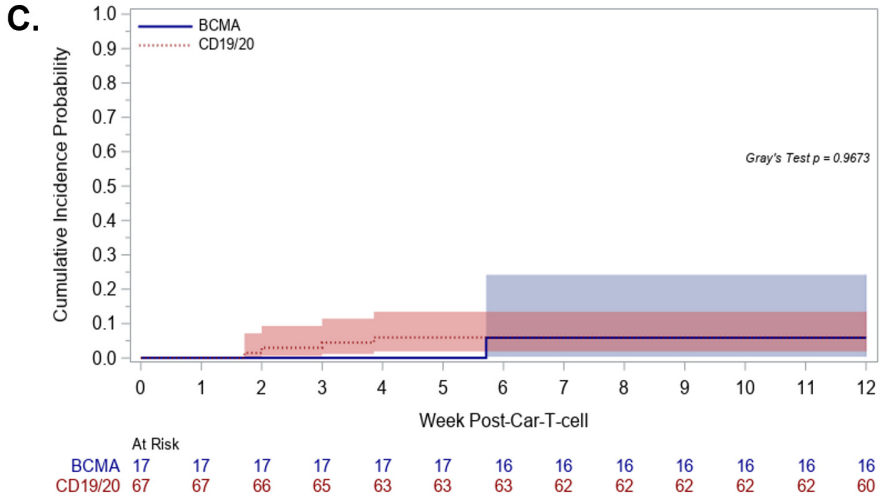
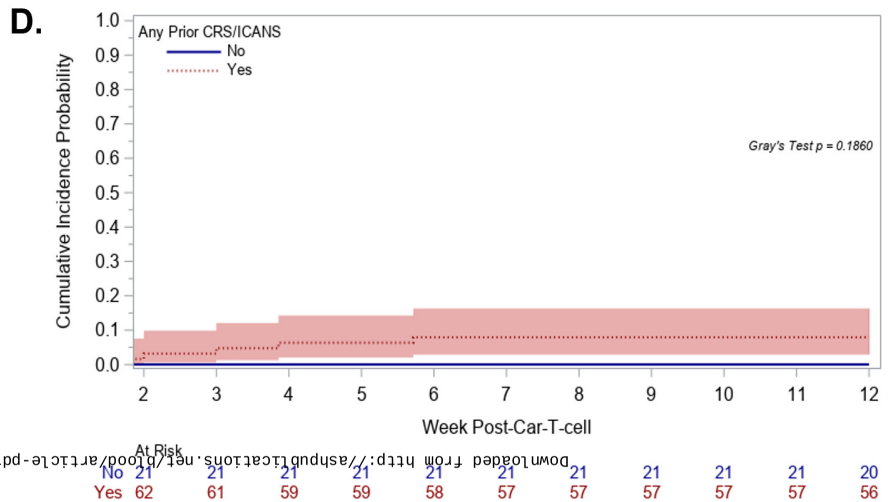
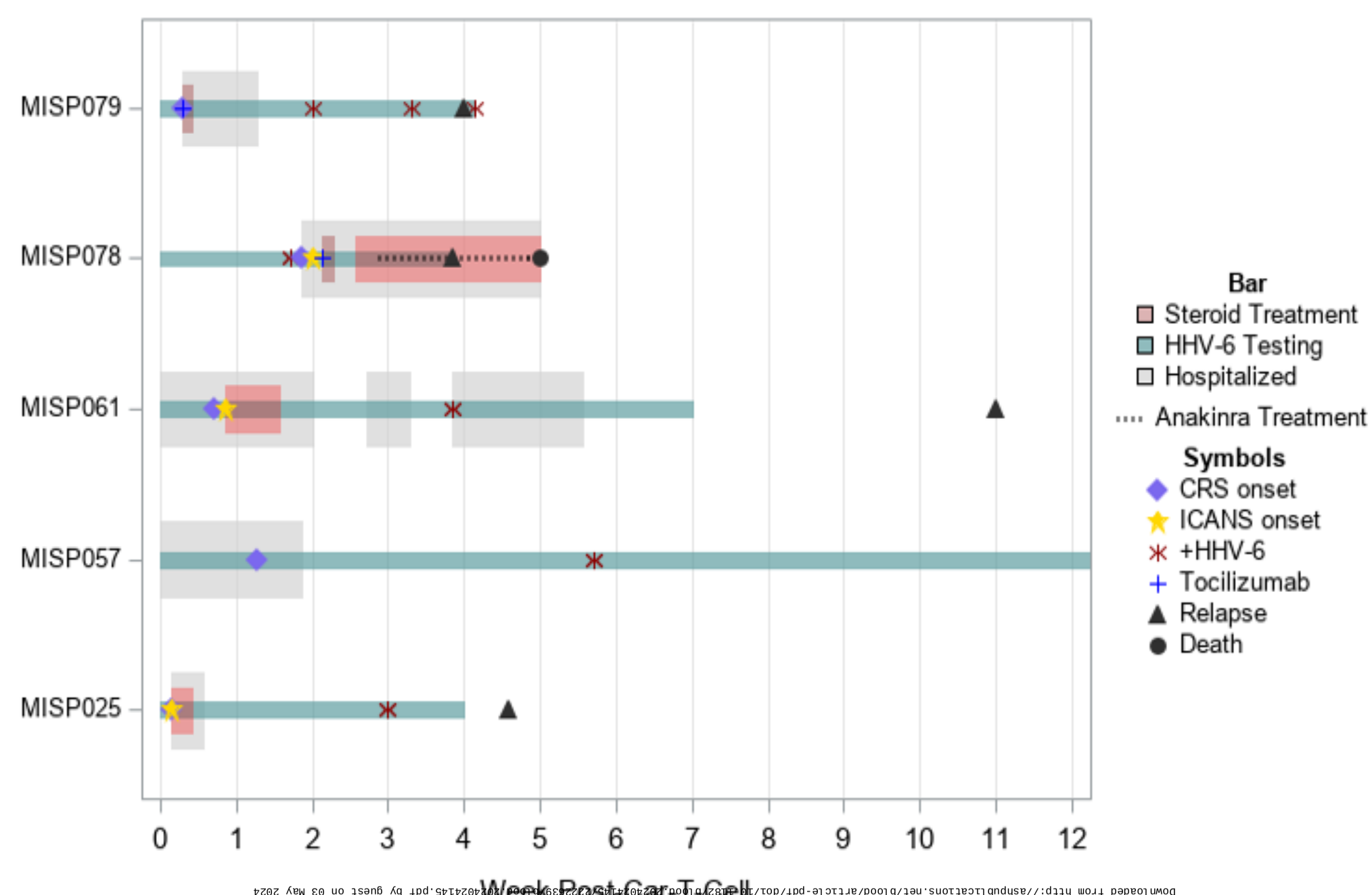
Figure 1





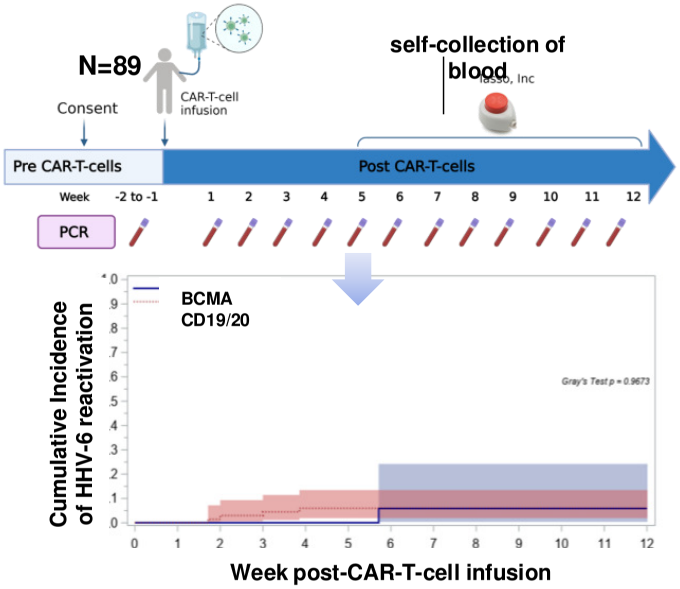






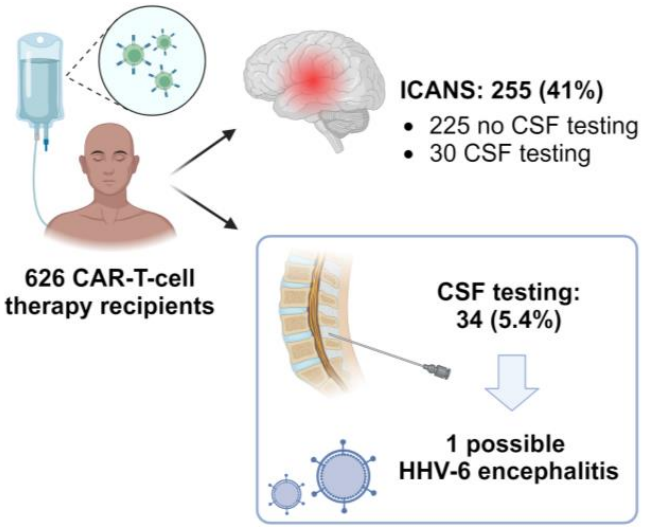
Incidence of Human Herpesvirus-6B (HHV-6B) Reactivation and Disease in Recipients of Chimeric Antigen Receptor (CAR)-T Cell Therapy

Prospective Study



HHV-6 reactivation occurred in 6% (95% confidence interval, 2.2-12.5%) of participants within 12 weeks after infusion

Retrospective Study



The estimated cumulative incidence of HHV-6B encephalitis was 0.17% (95% confidence interval, 0.02-0.94%) within 12 weeks after infusion

Conclusions: 1) HHV-6 reactivation and disease are infrequent after CAR-T-cell therapy. 2) Routine HHV-6 monitoring is not warranted.

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Blood Visual Abstract