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Final Outcomes from a Phase 2 Trial of Posoleucel in Allogeneic Hematopoietic Cell Transplant Recipients

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Abstract:
Allogeneic hematopoietic cell transplantation (allo-HCT) recipients are susceptible to viral infections. We conducted a phase 2 trial evaluating the safety and rate of clinically significant infections (CSIs; viremia requiring treatment or end-organ disease) following infusion of posoleucel, a partially HLA-matched, allogeneic, off-the-shelf, multivirus-specific T cell investigational product for preventing CSIs with adenovirus, BK virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus-6, or JC virus. This open-label trial enrolled high-risk allo-HCT recipients based on receiving grafts from umbilical cord blood, haploidentical, mismatched, or matched unrelated donors; post-HCT lymphocytes <180/mm3; or use of T cell depletion. Posoleucel dosing was initiated within 15-49 days of allo-HCT and subsequently every 14 days for up to seven doses. The primary endpoint was the number of CSIs due to the six target viruses by week 14. Of the 26 patients enrolled just three (12%) had a CSI by week 14, each with a single target virus. In vivo expansion of functional virus-specific T cells detected via interferon-γ ELISpot assay was associated with viral control. Persistence of posoleucel-derived T cell clones for up to 14 weeks after the last infusion was confirmed by T cell receptor deep-sequencing. Five patients (19%) had acute GVHD grade II-IV. No patient experienced cytokine release syndrome. All six deaths were due to relapse or disease progression. High-risk allo-HCT patients who received posoleucel had low rates of CSIs from six targeted viruses. Repeat posoleucel dosing was generally safe and well tolerated and associated with functional immune reconstitution. www.clinicaltrials.gov NCT04693637.-

Conflict of interest: COI declared - see note

COI notes: Sanjeet Dadwal SD has served on an advisory board for Merck; has served as a speaker for Takeda, Merck, and Astellas; has received research funding from AlloVir, Karius, Ansun Biopharma, Merck, and Amplyx/Pfizer; and has stock options with Aseptiscope, Inc. G. Doug Myers has served on an advisory board and speakers bureau for Novartis, he has consulted for Eliana, and has received research funding from AlloVir. Jo-Anne Young, Rajat Bansal, Jean Yared have nothing to declare. Michelle Matzko, Sama Adnan, Sarah Gilmore are employees of and hold stock in AlloVir. Spyridoula Vasileiou and Ann Leen are consultants for AlloVir. Joshua Hill has served as a consultant for Amplyx.

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Short title: Posoleucel to Prevent Post-HCT Viral Infections

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Data Sharing Statement

Qualified researchers may request from AlloVir data supporting the clinical findings of this study by contacting info@allovir.com. Individual patient data will not be shared.

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Key Points

- Allogeneic hematopoietic cell transplant recipients are at risk of clinically significant viral infections due to lack of T cell immunity
- The multi-virus specific T cell therapy posoleucel appeared to reduce the risk of clinically significant viral infections

ABSTRACT

Allogeneic hematopoietic cell transplantation (allo-HCT) recipients are susceptible to viral infections. We conducted a phase 2 trial evaluating the safety and rate of clinically significant infections (CSIs; viremia requiring treatment or end-organ disease) following infusion of posoleucel, a partially HLA-matched, allogeneic, off-the-shelf, multivirus-specific T cell investigational product for preventing CSIs with adenovirus, BK virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus-6, or JC virus. This open-label trial enrolled high-risk allo-HCT recipients based on receiving grafts from umbilical cord blood, haploidentical, mismatched, or matched unrelated donors; post-HCT lymphocytes <180/mm^3; or use of T cell depletion. Posoleucel dosing was initiated within 15-49 days of allo-HCT and subsequently every 14 days for up to seven doses. The primary endpoint was the number of CSIs due to the six target viruses by week 14. Of the 26 patients enrolled just three (12%) had a CSI by week 14, each with a single target virus. In vivo expansion of functional virus-specific T cells detected via interferon-γ ELISpot assay was associated with viral control. Persistence of posoleucel-derived T cell clones for up to 14 weeks after the last infusion was confirmed by T cell receptor deep-sequencing. Five patients (19%) had acute GVHD grade II-IV. No patient experienced cytokine release syndrome. All six deaths were due to relapse or disease progression. High-risk allo-HCT patients who received posoleucel had low rates of CSIs from six targeted viruses. Repeat posoleucel dosing was generally safe and well tolerated and associated with functional immune reconstitution. www.clinicaltrials.gov NCT04693637.

INTRODUCTION

The past two decades have seen a steady rise in patients undergoing hematopoietic cell transplantation (HCT) from human leukocyte antigen (HLA) mismatched donors.[1-3] This increase in high-risk allogeneic HCT (allo-HCT) has been largely driven by the availability of mismatched donors and improvements in graft-vs-host disease (GVHD) prevention.[4] Newer T cell ablative and post-transplant cyclophosphamide (PTCy)-based regimens, which are becoming standard of care for most mismatched and matched unrelated donor transplants, have led to significant reductions in the rates of GVHD and non-relapse mortality (NRM).[5] As a result, allo-HCT is increasingly available to patients who had previously not been eligible because of age, lack of matched donor, frailty, or disease status.[6,7]

However, the high level of immune suppression required to overcome the HLA barrier is known to increase the risk of potentially severe opportunistic infections from double-stranded DNA viruses, including adenovirus (AdV), BK virus (BKV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus-6 (HHV-6), and JC virus (JCV).[8-9] In the first 100 days after allo-HCT, approximately 90% of patients have reactivation of one of these viruses, and over 60% have reactivations of more than one virus.[10,11] A nearly 40% increase in NRM is observed for every $\log_{10}$ increase of viral burden during the first 100 days post-allo-HCT.[10] The use of anti-thymocyte globulin (ATG) has been linked to an increased risk of EBV and HHV-6 reactivation and disease, while PTCy is known to increase the risk of BKV and CMV reactivation and disease.[12-16] Over 60% of these viral reactivations progress to clinically significant infections (CSIs; defined as viremia requiring antiviral treatment or end-organ disease), resulting in substantial morbidity and mortality.[10]

The therapeutic armamentarium against these viruses is limited.[17-19] There are no approved antivirals to treat AdV, BKV, EBV, JCV, and HHV-6, while the small-molecule antivirals used to treat or prevent CMV infection have suboptimal efficacy and carry the risk of severe toxicities and development of resistance.[20,21] The limitations of existing treatments have encouraged research on preventive approaches. Some of the same broad-spectrum antivirals used in treatment have been used as prophylaxis but with the same shortcomings. The CMV DNA terminase-complex inhibitor letermovir, which was approved in 2017 for the prevention of CMV infection after allo-HCT, has an improved safety profile and greater potency at preventing CMV replication than other antivirals, but it has a relatively low barrier to resistance and targets only CMV.[22] In the registrational phase 3 trial, letermovir had no statistically significant impact on all-cause mortality after allo-HCT,[11] and late reactivations after the cessation of letermovir are common in high-risk patients.[23] Moreover, letermovir, like other small-molecule antivirals, does not address the root cause of the heightened risk of viral infection—the absence of T cell immunity.

Adoptive virus-specific T cell therapy, which has been studied for the treatment and prophylaxis of dsDNA viral infections after allo-HCT, may offer an alternative approach to prevention without the disadvantages associated with small molecule antivirals.[24-30] Posoleucel is an allogeneic, off-the-shelf, multivirus-specific T cell therapy designed for administration as a partially HLA-matched product for the prevention or treatment of CSIs due to AdV, BKV, CMV, EBV, HHV-6, and JCV in immunocompromised patients. Posoleucel is polyclonal and polyfunctional with a low potential for alloreactivity (see Supplementary Appendix for further information).[31,32] In the phase 2 CHARMS treatment trial, 95% of allo-HCT recipients with refractory and resistant infections due to the targeted viruses who received posoleucel had a

partial or complete clinical response with no safety or tolerability concerns.[33,34] This successful outcome prompted the current phase 2 study designed to evaluate the safety of posoleucel and its potential to prevent viral reactivation or de novo infection from progressing to CSIs (with the six viruses as detailed above) in allo-HCT recipients.

METHODS

Eligibility criteria and study design

This open-label, single-arm, phase 2 study enrolled patients who were within 15 and 49 days of allo-HCT and who were at high risk for CSIs, defined as an AdV, BKV, CMV, EBV, HHV-6, or JCV viral infection requiring treatment. High-risk patients were defined as those who had received a graft from a haploidentical, mismatched unrelated, matched unrelated donor, or from umbilical cord blood , had lymphocytes $<180/mm^3$ at the time at which a partially HLA matched posoleucel cell line was identified (i.e. time of randomization) or patients who had received T cell depletion by ex vivo graft manipulation, ATG, or alemtuzumab. To be eligible, patients were required to have engrafted based on an absolute neutrophil count >500/μL. Those with grade ≥3 GVHD and those requiring high-dose steroids (>0.5 mg/kg/day prednisone equivalent) at enrollment were not eligible. All other immunosuppressive agents for GVHD prophylaxis were allowed. Viremia at screening was not exclusionary, but non-prophylactic receipt of antiviral therapy for a targeted virus or signs and symptoms of end-organ disease (EOD) from one of the targeted viruses in the prior 6 months were exclusionary. Standard-of-care antiviral prophylaxis was allowed, including letermovir. See Supplementary Appendix for full eligibility criteria. Investigators obtained informed consent from each participant or each participant's guardian. The study protocol, amendments, and informed consent forms were approved by the independent ethics committee or Institutional Review Board at participating centers. All authors had access to

primary clinical trial data. The study was conducted in accordance with the Declaration of Helsinki.

Posoleucel cell lines were selected for each patient based on a partial HLA match of at least 2 shared alleles between the transplant donor, recipient, and posoleucel cell line (see Supplementary Appendix for information on the manufacture of posoleucel and HLA alleles considered during the matching process). Each patient received infusions from the same cell line, which was generated from a single donor.

Patients received posoleucel once every 14 days (±3 days) for up to a total of 7 infusions, which were administered either in the hospital or in the infusion center. Cells were administered at a dose of 2×10^7 cells in 2 mL for patients <40 kg or 4×10^7 cells in 4 mL for patients ≥40 kg. For patients on ATG, alemtuzumab, or other immunosuppressive T cell-targeted monoclonal antibodies, posoleucel dosing was delayed by at least 28 days from the last dose of anti-T cell antibody.

AdV, BKV, CMV, EBV, HHV-6, and JCV viral loads were assessed by quantitative polymerase chain reaction (PCR) at a central lab during screening, weekly from the initiation of dosing through week 14, and then monthly through week 26. Viremia was defined as viral load above the lower level of quantitation. Patients were contacted by telephone or email approximately 52 weeks after their first treatment to assess one-year mortality if unknown from record review.

Endpoints

The primary endpoint of the study was the number of CSIs or EOD per patient due to AdV, BKV, CMV, EBV, HHV-6, or JCV through Week 14. A CMV CSI was defined as a viral load >910 IU/mL plus the initiation of antiviral therapy[35]. The definitions of CSIs of EBV or AdV

were viral loads >10,000 copies/mL (or two consecutive results of >1,000 copies/mL, with the second being higher than the first and drawn at least 48 hours after the first) plus the initiation of antiviral therapy. HHV-6, BKV, and JCV CSIs were not defined by viral load threshold, but by the development of associated EOD. EOD was defined as signature signs or symptoms of organ damage from AdV, BKV, CMV, EBV, HHV-6, or JCV. In the instance of end organ dysfunction where biopsy was not performed, assignment of cause was made by the study PI. The key secondary endpoint was the number of CSIs per patient through Week 26.

Safety endpoints included the severity and incidence of acute and chronic GVHD, cytokine release syndrome (CRS), and secondary graft failure. Clinical laboratory values, adverse events, and serious adverse events were assessed at 2-week intervals through week 26. Safety events were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.0.

Immunoassays

Interferon (IFN)-γ enzyme-linked immunosorbent spot (ELISpot) analysis was used to determine the frequency (spot-forming cells, SFC) of IFNγ+ producing T cells specific for target viruses.[36] Posoleucel is a non-gene-modified T cell product. Hence, to track the presence and persistence of posoleucel clones, TCRvβ sequencing (Adaptive Biotechnologies, Seattle, WA) was performed on the infused lines and serial patient peripheral blood samples collected before and after infusion.[37] T cell clones identified within posoleucel were compared with pre- and post-infusion patient samples. Those clones that were shared between posoleucel and the pre-infusion blood were non-discriminatory and not used for tracking, while those clones that were detected only in the post-infusion blood samples were defined as posoleucel-derived and used to assess

persistence. However, it is impossible to definitively discriminate between TCRs of endogenous vs posoleucel origin, since the latter is not genetically modified. Thus, the tracking approach (by TCRvβ deep sequencing and comparison with pre-infusion samples) may over- or underestimate the number of posoleucel-derived clones. TCR sequencing and ELISpot are complementary- TCRvβ sequencing tracks unique posoleucel-derived TCR sequences and IFNγ ELISpot detects functional, virus-specific T cells that are both endogenous and of posoleucel origin.

Statistical Analysis

CSIs were considered as a failure of prevention; thus, in a given patient, each virus was counted once, even if there were multiple episodes of viremia for a given virus. For the purposes of the primary analysis, only new-onset CSIs with one of the 6 target viruses that occurred after the patient's first dose of posoleucel were considered in the count of CSIs or episodes of EOD per patient.

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents were submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study was initiated.

RESULTS

Patient enrollment and disposition

Of the 37 patients screened for enrollment, 10 did not meet eligibility criteria. A matching posoleucel cell line was found for 36 of the 37 patients (97%) screened for enrollment. **Table 1** shows the demographics and baseline disease characteristics for the 26 patients enrolled and dosed. Most patients had received HCT grafts from haploidentical (n=12, 46%) or mismatched unrelated donors (n=9, 35%). Four patients (15%) had ATG, and 20 (77%) received PTCy. At

baseline, 12 patients (46%) had detectable viremia and 16 (62%) patients were receiving letermovir. Of the 11 patients screened but not dosed, 6 had baseline viral testing. Three patients (50%) had detectable viremia, one each with BKV, EBV, and JCV.

Dosing of the 26 patients began at a median of 42 days (range 23-52) after allo-HCT. The median number of posoleucel doses received was 7 (interquartile range 5-7). Of the 26 patients dosed, 16 completed treatment, and 10 discontinued dosing (**Supplementary Figure 1**). Four (15%) patients discontinued posoleucel due to one or more treatment-emergent adverse events (TEAEs) (see Safety section below).

Seventeen patients (65%) received systemic corticosteroids at doses >0.5 mg/kg/day (prednisone equivalent) at some point during the study, mostly in short courses to manage GVHD symptoms. Sixteen patients were on letermovir prophylaxis on Day 1; see CMV serologies in Table 1.

Primary and key secondary endpoints

In the first 14 weeks of posoleucel dosing (primary endpoint period), 23 of 26 patients (88%) remained free of CSIs from any of the target viruses with none developing hemorrhagic cystitis. Twenty-two of the 26 patients (85%) had viremia with one or more target viruses, and 13 (50%) had 2 or more viral reactivations (**Figure 1**). Only 3 patients (12%) progressed to CSIs. Two received preemptive valganciclovir for asymptomatic CMV viremia; both had been on letermovir previously. One patient developed EBV post-transplant lymphoproliferative disease (PTLD) 6 days after receiving a single dose of intravenous hydrocortisone (75 mg) to treat a sensitivity reaction and in the setting of ongoing methylprednisolone treatment for acute GVHD. All three patients with CSIs experienced rapid and full recovery (see **Supplementary Table 1** for details). No pattern was observed in outcome based on the number of HLA matches or HLA

match by class. All three CSIs through Week 14 occurred in patients who matched at both HLA Class I and Class II loci to posoleucel.

From the initiation of posoleucel dosing until the end of Week 26 (secondary endpoint period), a total of 19 patients (73%) remained free of CSIs from any of the target viruses. With respect to the four additional patients who developed CSIs between weeks 15 and 26, three were cases of asymptomatic CMV viremia in patients who were antibody seropositive, had previously received letermovir prophylaxis, and who required preemptive antiviral treatment (see **Supplementary Table 2**). Of these three patients, one was receiving dasatinib, which is known to be associated with CMV reactivation,[38,39] and another occurred six weeks after the last posoleucel infusion, which had ceased when the patient was identified as having relapsed disease (multiple myeloma) relapse that was treated with ixazomib. The fourth CSI in this period was AdV viremia in a patient with diarrhea but not diagnosed with target organ disease. This patient was treated with cidofovir and intravenous immune globulin, and the viremia cleared five weeks later. None of the late CSIs progressed to EOD, per treating physician.

T cell function and persistence

To determine whether posoleucel dosing and the presence or absence of detectable viremia influenced the circulating frequency of virus-reactive T cells (endogenous and posoleucel derived), cellular immune responses to five of the target viruses (AdV, BKV, CMV, EBV, HHV-6) during viremic events were evaluated by IFNγ ELISpot within the primary endpoint period. Prior to posoleucel dosing, most patients lacked detectable T cell activity against any of the target viruses. In the absence of viremia, the frequency of virus-specific T cells remained relatively static. In contrast, viral reactivation was associated with an increase in the frequency of

functional virus-specific T cells (**Figure 2, panel A**). To determine whether changes in the frequency of functional virus-specific responses were associated with antiviral benefit, pre- and post-infusion T cell responses were evaluated in correspondence to peak and Week 14 viral loads in patients who were viremic for a target virus up to Week 14. Of 39 viremia events across five viruses that did not result in a CSI (AdV, n= 3; BKV, n=15; CMV, n=3; EBV, n=12; HHV-6, n=6), there was a reduction in viral titers in 74% of cases (29/39) with a corresponding increase in the circulating frequency of virus-specific T cells for the reactivating virus in 72% of cases (28/39) (**Figure 2, panel B**).

To evaluate the presence and persistence of posoleucel, we performed tracking studies using TCR deep sequencing with specific focus on detecting sequences unique to the infused posoleucel cell lines. All patients evaluated by TCR sequencing (n = 25/25) had detectable posoleucel T cell clones (albeit at low levels ranging from 0.0005-0.2%) at one or more time points during the dosing period of the study (Week 1 to Week 14) with persistence for up to 14 weeks after the dosing period in 21/21 evaluable patients (**Figure 2, panel C**). **Figure 3** shows representative posoleucel persistence and ELISpot data for three patients with viremia from multiple viruses not requiring treatments [BKV, EBV, HHV-6 (**panels A, B**); BKV, EBV (**panels C, D**); or BKV, CMV, EBV, JCV (**panels E, F**)]. Supplementary Figure 2 shows three patients with CSIs [EBV PTLD (**panels A, B**), CMV CSI and BK, JCV viremia (**panels C, D**), CMV CSI and BKV viremia (**panels E, F**)]. In patients with viremia that did (n=3) (**Supplementary Figure 2**) or did not progress to CSI (n=3) (Figure 3), viral control was coincident with detection of posoleucel clones and expansion of functional T cells against the replicating virus(es).

Safety

All 26 (100%) patients had at least one adverse event (AE) during the study (**Table 2**). The most common AEs were diarrhea (in 62% of patients) and acute skin GVHD (38%). Abdominal pain, dyspnea, pain in extremity, tremor, or decreased weight each occurred in 23% of patients. Nineteen patients (73%) had at least one serious adverse event (SAE), of which three (12%) were considered treatment-related by the investigator. Four (15%) patients had an AE that led to discontinuation of study treatment, while none had an AE that led to discontinuation of the study. No renal toxicity or myelosuppression was observed.

Five patients (19%) had MAGIC grade II-IV treatment-emergent acute GVHD: three with grade II, and one each with grades III and IV (see **Supplementary Table 3**). In this small sample, no trend was observed between the incidence or severity of GVHD and the number of matched HLA alleles (representing the number of shared alleles between the HCT donor, recipient, and posoleucel cell line received).Twenty-one of 26 (81%) received PTCy, and among this cohort 2/21 (10%) developed Grade II-IV aGVHD, 0/21 (0%) developed Grade III-IV aGVHD, and 3/21 (14%) developed moderate to severe cGVHD. No patient experienced CRS.

At one year on study, non-relapse mortality after allo-HCT was 0% (**Figure 4**). Six patients died, all as a result of primary disease relapse). None of the deaths were considered related to treatment by the investigator, and none was attributed to GVHD or infection with one of the target viruses.

DISCUSSION

In the present study, designed to evaluate posoleucel's safety and ability to prevent CSIs from six targeted dsDNA viruses in a high-risk cohort of allogenic HCT recipients, only 12% of patients

had CSIs in the first 14 weeks after the initiation of posoleucel. Moreover, the rate of NRM we observed after one year on study was 0%. These results support the safety and tolerability of posoleucel. The efficacy of posoleucel is being investigated in a placebo-controlled Phase 3 trial for the prevention of CSIs from the six targeted viruses, for decreasing the use of myelotoxic and nephrotoxic antivirals, and for potentially contributing to broadly favorable immune protection after allo-HCT. Since managing CSIs in transplant recipients often requires prolonged hospitalization and complex interventions, posoleucel has the potential to reduce health resource utilization, with attendant clinical and economic benefits.[40,41]

As a frequent cause of CSIs in allo-HCT recipients, CMV has received much attention from researchers, which has led to the development of new options for CMV prevention and treatment. Although AdV, BKV, EBV, and HHV-6 viremias are monitored less consistently, CSIs caused by these viruses are nonetheless important causes of post-transplant morbidity and adverse outcomes, and account for substantial healthcare resource utilization.[42-43] Importantly, patients frequently experience sequential or simultaneous viral reactivations/infections with corresponding increases in morbidity and mortality.[10] In the present study, there were no CSIs involving HHV-6, BKV, or JCV for the duration of the study (>6 months), and the incidence of CSIs involving AdV and EBV was only 4% even though 13 patients (50%) were viremic with more than one virus and were therefore at higher risk of CSIs. However, definitive efficacy results will require assessment in the context of a randomized, placebo-controlled Phase 3 clinical trial. Most patients who were viremic for two or more viruses demonstrated evidence of functional immune reconstitution for multiple viruses during posoleucel dosing. Together these data highlight the clinical utility of a multivirus-targeted therapy to bridge the lymphopenic

period after allo-HCT and support the mode of action of posoleucel to expand in response to viremia and ultimately control infection, preventing progression to clinically significant disease.

Posoleucel is designed not to prevent the occurrence of viremia, but to prevent reactivated or new infections from progressing to CSIs. Thus, the mostly subclinical viremia observed in this study, at rates roughly comparable to those seen in historical studies, was not unexpected. Importantly, low-level antigen exposure can activate and stimulate the expansion of the infused posoleucel cells. As seen in studies of CMV prevention using conventional antivirals, restricting CMV replication appears to delay CMV-specific immune reconstitution by eliminating antigens necessary for supporting functional immune reconstitution. In a study of CMV-specific T cell reconstitution in patients receiving letermovir, the subpopulation of patients with subclinical CMV reactivation during prophylaxis had superior CMV-specific CD8+ and CD4+ T cell responses at the end of prophylaxis than patients with complete suppression of reactivation.[44]

Viremia in our patient population resulted in a selective expansion of T cells reactive against the replicating virus(es), with consequent reduction of viremia. To determine whether posoleucel could have contributed to this immune reconstitution, TCR sequencing was used to track unique posoleucel clones during the study. This analysis confirmed the presence and persistence of posoleucel throughout dosing and follow-up, and serial sampling within patients highlighted changes in sum frequencies of posoleucel clones during viral reactivation.

The results of this trial add to the considerable body of evidence that virus-specific T cell therapy is a safe and well-tolerated approach.[45] Despite receiving multiple infusions of posoleucel, no patient experienced CRS, and the rate of acute GVHD was in line with rates seen in previous studies in high-risk allo-HCT patients. In addition, the rates of aGVHD II-IV (10%) and aGVHD

III-IV (0%) for patients receiving PTCy were in line with recently reported cohorts (53.8% and 6.3%, respectively). Since posoleucel consists of virus-specific CD4+ and CD8+ memory T cells, its potential for alloreactivity is low. No renal toxicity or myelosuppression was observed.

Interpretation of the results of this trial is necessarily limited given the relatively small sample size, lack of a comparison group, and relatively late initiation of posoleucel dosing (a median of 42 days after allo-HCT). Dosing sooner after the allo-HCT may benefit patients in potentially preventing CSIs that occur earlier in these severely immunosuppressed patients and has been implemented at within 25 (+5 days) post HCT in the Phase 3 study.

In this open label Phase 2 study, posoleucel appeared safe and well tolerated in this diverse group of patients. A phase 3 randomized, placebo-controlled study of posoleucel for the prevention of infection or disease due to AdV, BKV, CMV, EBV, HHV-6, or JCV in high-risk adult and pediatric patients after allogeneic HCT is currently ongoing (clinicaltrials.gov NCT05305040).

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AUTHORSHIP

Contributions: M.M. and A.M.L. designed the study; S.S.D., R.B., M.W.S., J.A.Y., G.D.M., J.A.H., and J.H.Y. provided study materials or patients, S.S.D., R.B., M.W.S., J.A.Y., G.D.M., J.A.H., J.H.Y., J.M., M.M., S.A., S.A.G., S.V., and A.L. collected and assembled data; S.S.D., M.M., S.A., S.A.G., S.V., J.M., and A.M.L. analyzed and interpreted the data; M.M., D.M., S.A.G., and A.M.L. drafted the manuscript; and all authors approved the manuscript and agree to be accountable for all aspects of the work.

Conflict-of-interest disclosure: Sanjeet Dadwal SD has served on an advisory board for Merck; has served as a speaker for Takeda, Merck, and Astellas; has received research funding from AlloVir, Karius, Ansun Biopharma, Merck, and Amplyx/Pfizer; and has stock options with Aseptiscope, Inc. G. Doug Myers has served on an advisory board and speakers bureau for Novartis, he has consulted for Eliana, and has received research funding from AlloVir. Jo-Anne Young, Rajat Bansal, Jean Yared have nothing to declare. Michelle Matzko, Julie Ma, Sama Adnan, Sarah Gilmore, and David McNeel are employees of and hold stock in AlloVir. Spyridoula Vasileiou and Ann Leen are consultants for and hold stock in AlloVir. Joshua Hill has served as a consultant for Amplyx.

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482 **Table 1. Demographic And Disease Characteristics**

Characteristic	N=26
Sex, n (%)	
Male	14 (54)
Female	12 (46)
Median age, yrs. (range)	59.5 (14-76)
Race, n (%)	
Caucasian	18 (69)
Black or African American	3 (12)
Multiracial	1 (4)
Native Hawaiian or Other Pacific Islander	1 (4)
Unknown or unspecified	3 (12)
Ethnicity, n (%)	
Not Hispanic or Latino	19 (73)
Hispanic or Latino	7 (27)
Diagnosis, (%)	
Acute myeloid leukemia	8 (31)
Acute lymphoblastic leukemia	5 (19)
Chronic myeloid leukemia	2 (8)
Chronic lymphocytic leukemia	1 (4)
Myelodysplasia/Myeloproliferative	3 (12)
Sickle cell anemia	2 (8)
Multiple myeloma	1 (4)
Other*	4 (15)
Transplant type, n (%)	
Haploidentical	12 (46)
Mismatched unrelated	9 (35)
Matched unrelated with T cell depletion	4 (15)
Umbilical cord blood	1 (4)
Preconditioning type, n (%)	
Myeloablative	12 (46)
Reduced intensity/non-myeloablative	14 (54)
CMV Donor/Recipient Serostatus, n (%)	
D-/R+	7 (27)
D+R+	9 (35)
D+/R-	3 (12)
D-/R-[$]	7 (27)
Receiving letermovir at baseline, n (%)	16 (62)
GVHD prophylaxis, n (%)	
ATG	4 (15)
PTCy	21 (81)
Viremia at Study Day 1, n (%)[†]	

BKV	8 (31)
HHV-6	5 (19)
EBV	2 (8)
Adv	1 (4)
None	14 (54)

*Adrenoleukodystrophy, cutaneous gamma-delta t-cell lymphoma, diffuse large B cell lymphoma, T cell prolymphocytic leukemia

[$]One patient was reported as CMV D-/R- but had CMV viremia. Upon investigation by the site, the patient was previously reported as R+ but was presumed to have lost their CMV antibody positivity in a prior CAR-T process.

[†]Eight patients had viremia with a single virus (BKV n=4, HHV-6 n=3, EBV n=1) and 4 had viremia with two viruses (BKV+HHV-6 n=2, AdV+BKV n=1, BKV+EBV n=1)

Table 2. Adverse Events, n (%)

Event	N=26
Patients with any treatment-emergent adverse event (AE)	26 (100)
Any treatment-related AE	12 (46)
Patients with most common treatment-emergent AEs (>20%)	
Diarrhea	16 (62)
Acute graft versus host disease in skin	10 (38)
Abdominal pain	6 (23)
Dyspnea	6 (23)
Pain in extremity	6 (23)
Tremor	6 (23)
Weight decreased	6 (23)
Any serious AE	19 (73)
Any treatment-related serious AE	3 (12)
Acute graft versus host disease in skin	1 (4)
Chronic graft versus host disease in lung	1 (4)
Hypersensitivity	1 (4)
Any grade ≥3 AE	19 (73)
Any treatment-related grade ≥3 AE	4 (15)
Any AE leading to discontinuation of study treatment	4 (15)
Pancreatitis	1 (4)
Acute graft versus host disease in skin	1 (4)
Graft versus host disease in gastrointestinal tract	1 (4)
Dyspnea	1 (4)
Any deaths related to treatment	0

Figure 1. Viremia and CSIs up to Week 14. Legend: For each numbered patient on study (N = 26), there are 6 rows, one per target virus. Bars represent the duration of measured viremia (viral load > LLOQ) per virus. The first panel shows all viremia including viremia that was categorized as CS or causing end-organ disease, the second panel shows duration of viremia categorized as clinically significant (2 patients with CMV), the third panel shows duration of viremia categorized as causing end-organ disease (1 patient with EBV). Nine of the 22 patients with viremia had detection of one target virus, five with two viruses, six with three viruses, and two with four viruses. Fifteen of 26 patients (58%) had detectable BKV by Week 14, making it the most frequently detected virus, followed by EBV (13/26 patients, 50%), HHV-6 (6/26 patients, 23%), CMV (5/26 patients, 19%), AdV (3/26 patients, 12%), and JCV (3/26 patients,

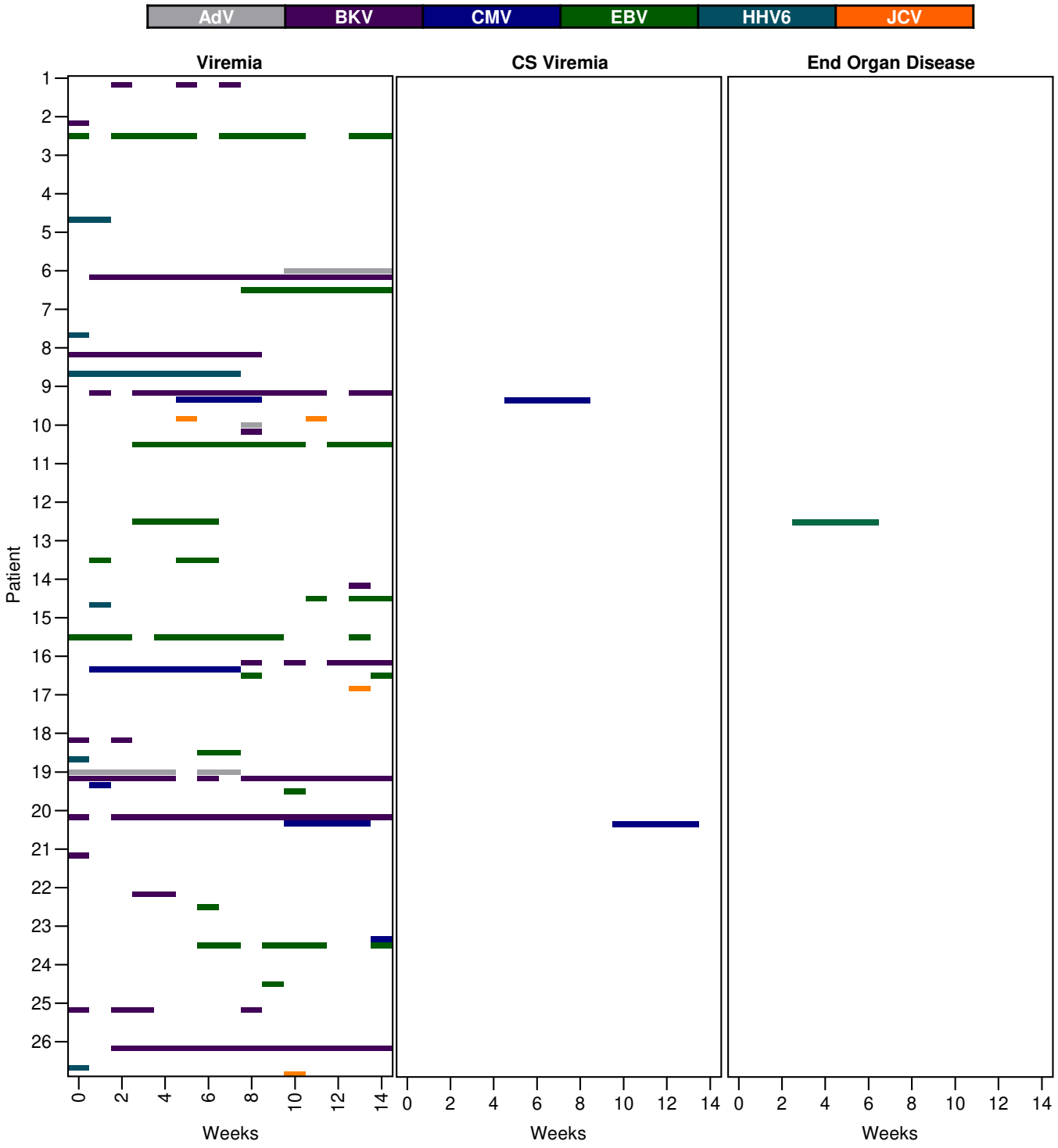
12%). Notably, there were no CSIs from BKV, HHV-6, or JCV, and a single EBV-associated CSI leading to end-organ disease. CS, clinically significant.

Figure 2. Viral load, Functional Virus-Specific Immune Reconstitution, and Posoleucel Persistence Functional immune reconstitution was evaluated by ELISpot assay. The frequency of virus- specific IFNγ+-producing cells (posoleucel and endogenous derived) was evaluated following stimulation of patient PBMCs with AdV, BKV, CMV, EBV, or HHV-6 antigens (SFC per 5 x 105 PBMCs). (A) ELISpot responses are plotted for all patients (and target viruses) with evaluable data prior to infusion (Pre; N = 53; mean = 14 SFC), that were aviremic for a target virus through Week 14 (Aviremic; N = 51; mean = 28 SFC), and that were viremic for a target virus through Week 14 (Viremic, N=39 out of a total of 45 viremic events; mean = 171 SFC). Box plots show the median with all values plotted. (B) ELISpot responses are plotted for all patients with evaluable data that were viremic for one or more viruses during the primary endpoint. Data shown represent the circulating frequency of IFNγ+ T cells prior to posoleucel infusion (Pre) and the peak response through Week 14 (Post). Viral loads are also shown per virus by plotting the peak viral load through Week 14 (Peak VL) and at Week 14/last time point available (Wk 14 VL). Viral loads from 3 patients with CSIs are excluded (2 CMV, 1 EBV). (C) TCRβ sequencing was used to track presence of TCRβ sequences unique to posoleucel during the infusion period and after (post-infusion). The percentage of patients with detectable posoleucel T cells during each indicated study period are shown. CSI, clinically significant infection; PBMC, peripheral blood mononuclear cells; SFC, spot- forming cells, VL, viral load.

Figure 3. Detection of Functional Immune Reconstitution and Posoleucel Clones Over Time Coincident with Viremia Reduction Patient examples of viral load plotted with unique posoleucel clones detected by TCRβ sequencing (left panels) and functional IFNγ+ virus-specific T cell responses detected by ELISpot (posoleucel and endogenous derived; right panels) through Week 26 of the study for patients with viremia of one or more target virus(es). Three patients with viremia that did not progress to CSI are shown: patient #1: BKV, EBV, and HHV-6 viremia (A, B; received all 7 doses of posoleucel); patient #2: BKV and EBV viremia (C, D; received all 7 doses of posoleucel); and patient #3: BKV, CMV, EBV, and JCV viremia (E, F; received all 7 doses of posoleucel). TCRβ clones unique to posoleucel are shown as the log2 fold change of the sum frequency of clones relative to first timepoint detected. Virus-specific IFNγ+-producing cells were measured by ELISpot after stimulation of patient PBMCs with AdV, BKV, CMV, EBV, or HHV-6 antigens (SFC per 5 x 105 PBMCs). All detectable viremia (viremia > LLOQ) is shown in the left panels with TCRβ sequencing data. In right panels with ELISpot data, only viremia for which there was corresponding ELISpot data is shown

Figure 4. Overall Survival through Week 52

Figure 1. Viremia and CSIs up to Week 14



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Legend: For each numbered patient on study (N = 26), there are 6 rows, one per target virus. Bars represent the duration of measured viremia (viral load > LLOQ) per virus. The first panel shows all viremia including viremia that was categorized as CS or causing end-organ disease, the second panel shows duration of viremia categorized as clinically significant (2 patients with CMV), the third panel shows duration of viremia categorized as causing end-organ disease (1 patient with EBV). Nine of the 22 patients with viremia had detection of one target virus, five with two viruses, six with three viruses, and two with four viruses. Fifteen of 26 patients (58%) had detectable BKV by Week 14, making it the most frequently detected virus, followed by EBV (13/26 patients, 50%), HHV-6 (6/26 patients, 23%), CMV (5/26 patients, 19%), AdV (3/26 patients, 12%), and JCV (3/26 patients, 12%). Notably, there were no CSIs from BKV, HHV-6, or JCV, and a single EBV-associated CSI leading to end-organ disease. CS, clinically significant.

Figure 2. Viral load, Functional Virus-Specific Immune Reconstitution, and Posoleucel Persistence

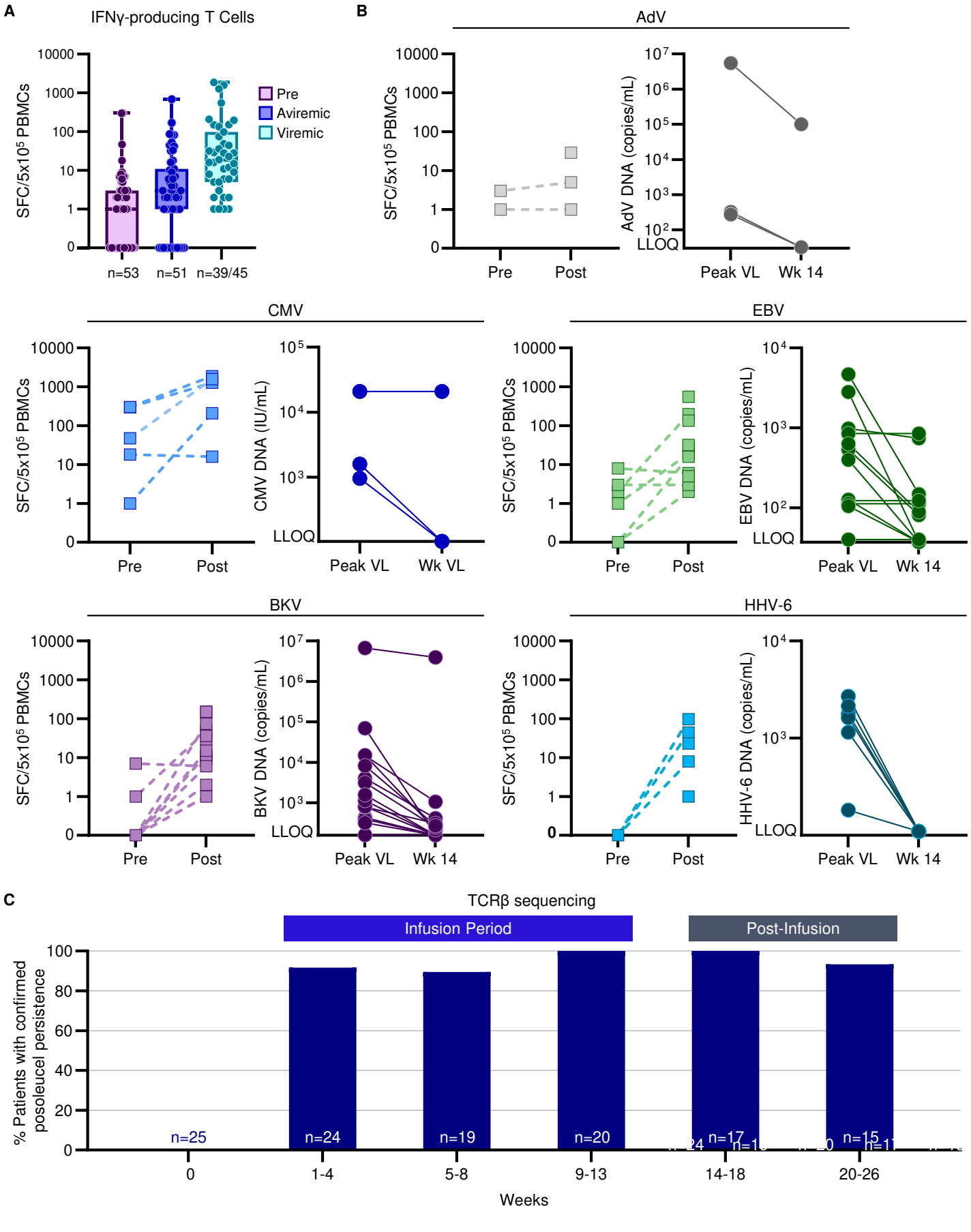

Figure 2. Viral load, Functional Virus-Specific Immune Reconstitution, and Posoleucel Persistence

Figure 2 Legend:

Functional immune reconstitution was evaluated by ELISpot assay. The frequency of virus-specific IFNγ+-producing cells (posoleucel and endogenous derived) was evaluated following stimulation of patient PBMCs with AdV, BKV, CMV, EBV, or HHV-6 antigens (SFC per 5 x 10^5 PBMCs). (A) ELISpot responses are plotted for all patients (and target viruses) with evaluable data prior to infusion (Pre; N = 53; mean = 14 SFC), that were aviremic for a target virus through Week 14 (Aviremic; N = 51; mean = 28 SFC), and that were viremic for a target virus through Week 14 (Viremic, N=39 out of a total of 45 viremic events; mean = 171 SFC). Box plots show the median with all values plotted. (B) ELISpot responses are plotted for all patients with evaluable data that were viremic for one or more viruses during the primary endpoint. Data shown represent the circulating frequency of IFNγ+ T cells prior to posoleucel infusion (Pre) and the peak response through Week 14 (Post). Viral loads are also shown per virus by plotting the peak viral load through Week 14 (Peak VL) and at Week 14/last time point available (Wk 14 VL). Viral loads from 3 patients with CSIs are excluded (2 CMV, 1 EBV). (C) TCRβ sequencing was used to track presence of TCRβ sequences unique to posoleucel during the infusion period and after (post-infusion). The percentage of patients with detectable posoleucel T cells during each indicated study period are shown.
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Figure 3. Detection of Functional Immune Reconstitution and Posoleucel Clones Over Time Coincident with Viremia Reduction

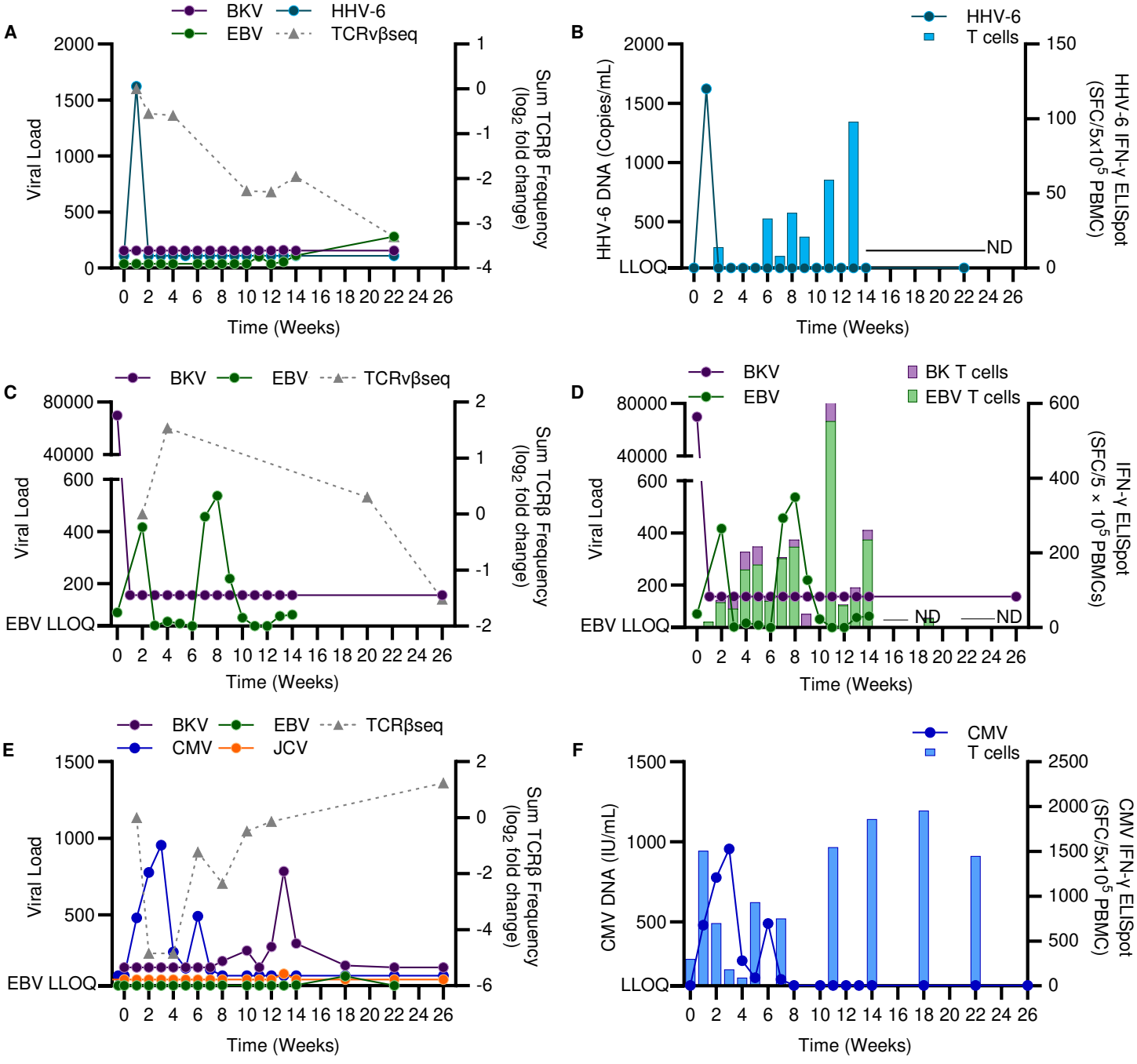

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Figure 3 Legend:

Patient examples of viral load plotted with unique posoleucel clones detected by TCRβ sequencing (left panels) and functional IFNγ+ virus-specific T cell responses detected by ELISpot (posoleucel and endogenous derived; right panels) through Week 26 of the study for patients with viremia of one or more target virus(es). Three patients with viremia that did not progress to CSI are shown: patient #1: BKV, EBV, and HHV-6 viremia (A, B; received all 7 doses of posoleucel); patient #2: BKV and EBV viremia (C, D; received all 7 doses of posoleucel); and patient #3: BKV, CMV, EBV, and JCV viremia (E, F; received all 7 doses of posoleucel). TCRβ clones unique to posoleucel are shown as the log2 fold change of the sum frequency of clones relative to first timepoint detected. Virus-specific IFNγ+-producing cells were measured by ELISpot after stimulation of patient PBMCs with AdV, BKV, CMV, EBV, or HHV-6 antigens (SFC per 5 x 105 PBMCs). All detectable viremia (viremia > LLOQ) is shown in the left panels with TCRβ sequencing data. In right panels with ELISpot data, only viremia for which there was corresponding ELISpot data is shown

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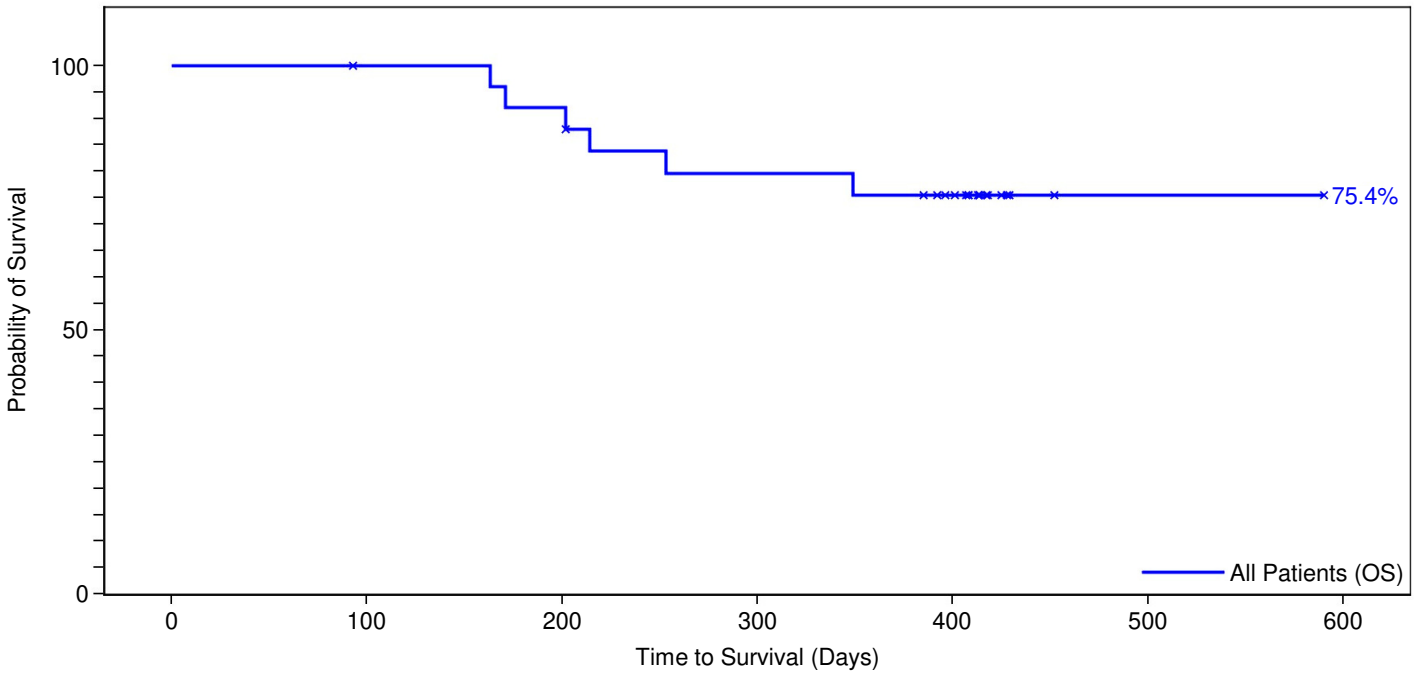

Number at Risk:

Posoleucel	26	25	23	19	15	1