Supplementary Appendix

Supplement to: Dadwal SS, Bansal R, Schuster MW, et al. Posoleucel to Prevent Clinically Significant Viral Infections in High-Risk Patients after Allogeneic Hematopoietic Cell Transplant: Results of an Open-Label Phase 2 Trial

CONTENTS

Posoleucel Manufacturing

Posoleucel is manufactured from peripheral blood mononuclear cells (PBMCs) collected from healthy, third-party donors. These donors have been selected to cover as broadly as possible the HLA diversity of the population at large. Existing posoleucel banks have cell lines matched at a minimum of 2 HLA alleles (matched alleles are defined as shared between the patient, transplant, and posoleucel cell line) estimated to cover 95% of the global population. The HLA alleles used for evaluation of matching were HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1. Each patient received infusions from the same cell line, which was generated from a single donor. PBMCs are cultured with growth-promoting cytokines and viral peptides spanning immunogenic antigens from each of the target viruses: AdV (Hexon and Penton), BKV (VP1 and Large T), CMV (IE1 and pp65), EBV (LMP2, EBNA1, BZLF1) and HHV6 (U90, U11, and U14). After manufacture, posoleucel T cell function is evaluated by IFNγ ELISpot assay using ADV, BKV, CMV, EBV, and HHV-6 antigens and posoleucel is cryopreserved for distribution.

Complete Eligibility Criteria

Inclusion Criteria

Age

- Be ≥1 year of age at the day of screening.

Type of Participant and Disease Characteristics

- Has no evidence of AdV, BKV, CMV, EBV, HHV-6, and JCV viremia from a central laboratory at any time prior to treatment assignment OR has evidence of AdV, BKV, CMV, EBV, HHV-6, and/or JCV viremia without symptoms or disease, from a central laboratory at any time prior to treatment assignment

- Be within 15 and 42 (+7) days of receiving a first allogeneic HCT at the time of treatment assignment and have demonstrated engraftment with an absolute neutrophil count >500/μL.

- High-risk: Patients meeting one or more of the following criteria at the time of treatment assignment:

o Human leukocyte antigen (HLA)-related (sibling) donor with at least one mismatch at one of the following three HLA-gene loci: HLA-A, -B or -DR

o Haploidentical donor

o Unrelated donor with at least one mismatch at one of the following four HLA-gene loci: HLA-A, -B, -C and -DRB1

o Use of umbilical cord blood as stem cell source

o Ex vivo graft manipulation resulting in T cell depletion

o Lymphocyte count <180/mm3 and/or cluster of differentiation 4 (CD4) T cell count <50/mm3

Sex

- Male and/or female

a. Male participants:

Male participants are eligible to participate if they agree to the following during the study intervention period and for at least 90 days after the last dose of study intervention:

• Refrain from donating sperm PLUS, either:

• Be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

OR

• Must agree to use contraception /barrier as detailed below

• Agree to use a male condom and should also be advised of the benefit for a female partner to use a highly effective method of contraception as a condom may break or leak when having sexual intercourse with a woman of childbearing potential (WOCBP) who is not currently pregnant

b. Female participants:

• A female participant is eligible to participate if she is not pregnant or breastfeeding, and 1 of the following conditions applies:

• Is a woman of non-childbearing potential (WONCBP) as defined in Section 10.4.1

OR

• Is a WOCBP and using an acceptable contraceptive method as described in Section 10.4.2 during the study intervention period and for at least 90 days after the last dose of study intervention. The Investigator should evaluate the potential for contraceptive method failure (eg, noncompliance, recently initiated) in relationship to the first dose of study intervention.

• A WOCBP must have a negative highly sensitive serum pregnancy test within 14 days before the first dose of study intervention, see Section 8.4.5.

Informed Consent

-Willing and able to provide written informed consent as described in Section 10.1.3 to participate in the study, or a parent or legal guardian is willing and able to provide written informed consent and the potential pediatric patient is able to provide assent in a manner

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from participation in the study:

Medical Conditions

1. Has a history of AdV, BKV, CMV, EBV, HHV-6, and/or JCV end-organ disease within 6 months prior to treatment assignment

2. Has evidence of AdV, BKV, CMV, EBV, HHV-6, and/or JCV viremia for more than 3 viruses, from a central or local laboratory at any time during Screening prior to treatment assignment

3. Evidence of active Grade >2 acute GVHD (for additional information on acute GVHD grading and severity, see Appendix 5 [Section 10.5] or CRS Appendix 6 [Section 10.6], respectively).

4. Presence of non-minor uncontrolled or progressive bacterial or fungal infections (ie, evidence of bacteremia, fungemia, disseminated, and/or organ-specific infection not well controlled by present therapies)

5. Presence of progressive, uncontrolled viral infections with evidence of end organ disease

6. Known history or current (suspected) diagnosis requiring treatment of CRS associated with the administration of peptides, proteins, and/or antibodies

7. Evidence of encephalopathy at screening

8. Relapse of primary malignancy other than minimal residual disease.

Prior/Concomitant Therapy

9. Donor lymphocyte infusion performed within 21 days prior to randomization

10. Received within 7 days prior to treatment assignment any of the following: ganciclovir, valganciclovir, foscarnet, acyclovir (at doses >3200 mg PO per day or >25 mg/kg IV per day), valacyclovir (at doses >3000 mg PO per day), famciclovir (at doses >1500 mg PO per day)

11. Received any investigational antiviral agent/biologic therapy within 30 days prior to screening or plans to receive during the study any of the following: cidofovir, CMV hyper-immune globulin, or any investigational CMV antiviral agent/biologic therapy.

12. Ongoing therapy with high-dose systemic corticosteroids (ie, prednisone equivalent dose

>0.5 mg/kg/day) within 7 days prior to screening

13. Prior therapy with antithymocyte globulin, alemtuzumab (Campath-1H), or other immunosuppressive T cell-targeted monoclonal antibodies within 28 days of treatment assignment

14. Receipt of mechanical ventilation of any type, within 1 month prior to the administration of ALVR105 (unless related to airway control)

15. Undergoing dialysis at any time during the screening period

Prior/Concurrent Clinical Study Experience

16. Received a previous allogeneic HCT (Note: Receipt of a previous autologous HCT is acceptable)

17. Receipt of another investigational antiviral vaccine or treatment during the study or within 28 days prior to treatment assignment or study treatment administration

Diagnostic assessments

18. Aspartate aminotransferase or alanine aminotransferase serum levels >5 × the upper limit of normal (ULN) or direct bilirubin serum levels >2 × the ULN reference per central laboratory.

19. Presence of any progressive, uncontrolled viral infections (ie, evidence of viremia, dissemination, and/or organ-specific infection not well controlled by present therapies) not targeted by ALVR105.

Other Exclusions

20. Pregnant, breastfeeding, or planning to become pregnant during the study

21. Has a history or current evidence of any condition, therapy, lab abnormality, or other circumstance that might confound the results of the study, interfere with the patient's participation for the full duration of the study, or would be put at undue risk as judged by the Investigator, such that it is not in the best interest of the patient to participate in this study.

Supplementary Table 1. CSIs in in Weeks 1-14 (Primary Endpoint)

Patient details	CMV Viral Load	CSI Start/End Day	Treatment Start/End Day	Relevant Conmeds
Age/sex: 66/M Disease: Diffuse LCL Donor type: PBSC, haplo Matched HLA alleles: 3 Conditioning: Myeloablative, TBI GVHD prophylaxis: PTCy Donor/Recipient CMV serostatus: -/- Letermovir at BL: Yes Viremia on Day 1: BKV 367 copies/mL	Week -1: 94.8 copies/mL			**LTV Day -27 to 16**
	Weeks 1 thru 9: <LLOQ			
	Week 10: 571.3 copies/mL			
	Week 11: 8,647.2 copies/mL	**CMV CSI Day 79-100**	**Valganciclovir Day 79-99**	
	Week 12: 2,863.4 copies/mL			
	Week 13: 404.5 copies/mL			
	Week 14: <LLOQ			
	Week 18: 217.2 copies/mL			
	Week 22: 1,686.1 copies/mL		**Valganciclovir Day 141-**	
	Week 26: <LLOQ			
Age/sex: 57/F Disease: Diffuse CLL Donor type: PBSC, MMUD Matched HLA alleles: 4 Conditioning: RIC GVHD prophylaxis: Tacrolimus Donor/Recipient CMV serostatus: +/+ Letermovir at BL: Yes Viremia on Day 1: No	**CMV Viral Load**	**CSI Start/End Day**	**Treatment Start/End Day**	**Relevant Conmeds**
	Weeks 1 thru 4: <LLOQ			**LTV Day -34 to 15**
	Week 5: 1,686.1 copies/mL	**CMV CSI Day 37-64**	**Valganciclovir Day 37-118**	
	Week 6: 1,686.1 copies/mL			
	Week 7: 1,435.1 copies/mL			
	Week 8: 104.0 copies/mL			
	Week 9 thru ET: <LLOQ			
Age/sex: 14/F Disease: Diffuse mL Donor type: Bone marrow, haplo Matched HLA alleles: 2 Conditioning: Myeloablative GVHD prophylaxis: PTCy Donor/Recipient CMV serostatus: +/- Letermovir at BL: No Viremia on Day 1: No	**EBV viral Load**	**CSI Start/End Day**	**Treatment Start/End Day**	**Relevant Conmeds**
	Weeks 1 thru 2: <LLOQ			
	Week 3: 120 copies/mL			
	Week 4: 257 copies/mL			**Hydrocortisone (1 dose 75 mg IV) Day 28**
	Week 5: 6,764 copies/mL			
	Week 6: 64,595 copies/mL	**EBV CSI Day 37-49**	**Rituximab Day 37**	**Methylprednisolone (47 mg IV) Day 37**
	Weeks 7 thru ET: <LLOQ			
			Rituximab Day 44	**Methylprednisolone (46 mg IV) Day 44**

Note: Weeks and Days are in relation to the initiation of posoleucel dosing.

Supplementary Table 2. CSIs in Weeks 15-26

Patient details	AdV viral load	CSI Start/End Day	Treatment Start/End Day	Relevant Conmeds		
Age/sex: 49/F Disease: Diffuse AML Donor type: PBSC, haplo Matched HLA alleles: 3 Conditioning: RIC GVHD prophylaxis: Tacrolimus Donor/Recipient CMV serostatus: -/+ Letermovir at BL: Yes* Viremia on Day 1: No *Patient receiving LTV throughout study	Weeks 1 thru 9: <LLOQ			LTV Day -138 to 88	Methyl-prednisolone (55 mg IV) Day 8	Prednisone taper (60-10 mg) Day 20-58
	Week 10: 41 copies/mL					
	Week 11: 134,896 copies/mL					
	Week 12: 5,495,409 copy/mL					
	Week 13: 69,183 copies/mL					
	Week 14: 100,000 copies/mL	**AdV Day 103-129**	**Cidofovir Day 103-147**			
	Weeks 18 thru ET: <LLOQ					
Age/sex: 31/F Disease: Diffuse ALL Donor type: PBSC, haplo Matched HLA alleles: 4 Conditioning: Myeloablative, TBI GVHD prophylaxis: PTCy Donor/Recipient CMV serostatus: +/+ Letermovir at BL: Yes Viremia on Day 1: AdV 240 copies/mL	**CMV viral load**	**CSI Start/End Day**	**Treatment Start/End Day**	**Relevant Conmeds**		
	Week -2: <LLOQ			**Letermovir Day -35 to 9**		
	Week -1: 80.7 copies/mL					
	Day 1: <LLOQ					
	Week 1: 1,016.0 copies/mL		**Valganciclovir Day 9**			
	Weeks 2 thru 14: <LLOQ					
				Letermovir Day 29-71		
	Week 18: 702.9 copies/mL	**CMV Day 143-155**	**Valganciclovir Day 143-162**	**Dasatinib Day 107 (ongoing)**		
	Week 22: <LLOQ					
	Week 26: 249.4 copies/mL					
Age/sex: 51/M Disease: Diffuse AML Donor type: PBSC, haplo Matched HLA alleles: 2 Conditioning: Myeloablative GVHD prophylaxis: MMF Donor/Recipient CMV serostatus: -/+ Letermovir at BL: Yes Viremia on Day 1: No	**CMV viral load**	**CSI Start/End Day**	**Treatment Start/End Day**	**Relevant Conmeds**		
	Weeks 1 thru 14: <LLOQ			**Letermovir Day -35 to 127**		
	Week 18†: 545.6 copies/mL					
	Week 19: 7,028.7 copies/mL	**CMV Day 132-140***	**Valganciclovir Day 132-139**			
	Week 22: 1,166.5 copies/mL					
Age/sex: 41/F Disease: Diffuse MM Donor type: Bone marrow, MMUD Matched HLA alleles: 2 Conditioning: Myeloablative GVHD prophylaxis: Tacrolimus Donor/Recipient CMV serostatus: -/+ Letermovir at BL: Yes Viremia on Day 1: No	**CMV viral load**	**CSI Start/End Day**	**Treatment Start/End Day**	**Relevant Conmeds**		
	Weeks 1 thru 12: <LLOQ			**Letermovir Day -25 to 6**		
				Ixazomib citrate Day 63-70		
	Week 14: 13,392.9 copies/mL	**CMV Day 100-108***	**Ganciclovir Day 100-ongoing**			
	Week 15: 232.7 copies/mL					

Note: Weeks and Days are in relation to the initiation of posoleucel dosing.
*Not possible to assess end since no further viral loads
†No viral load data from Day 96 to 124.

Supplementary Table 3. Cases of Treatment-Emergent Acute GVHD (grade II-IV)

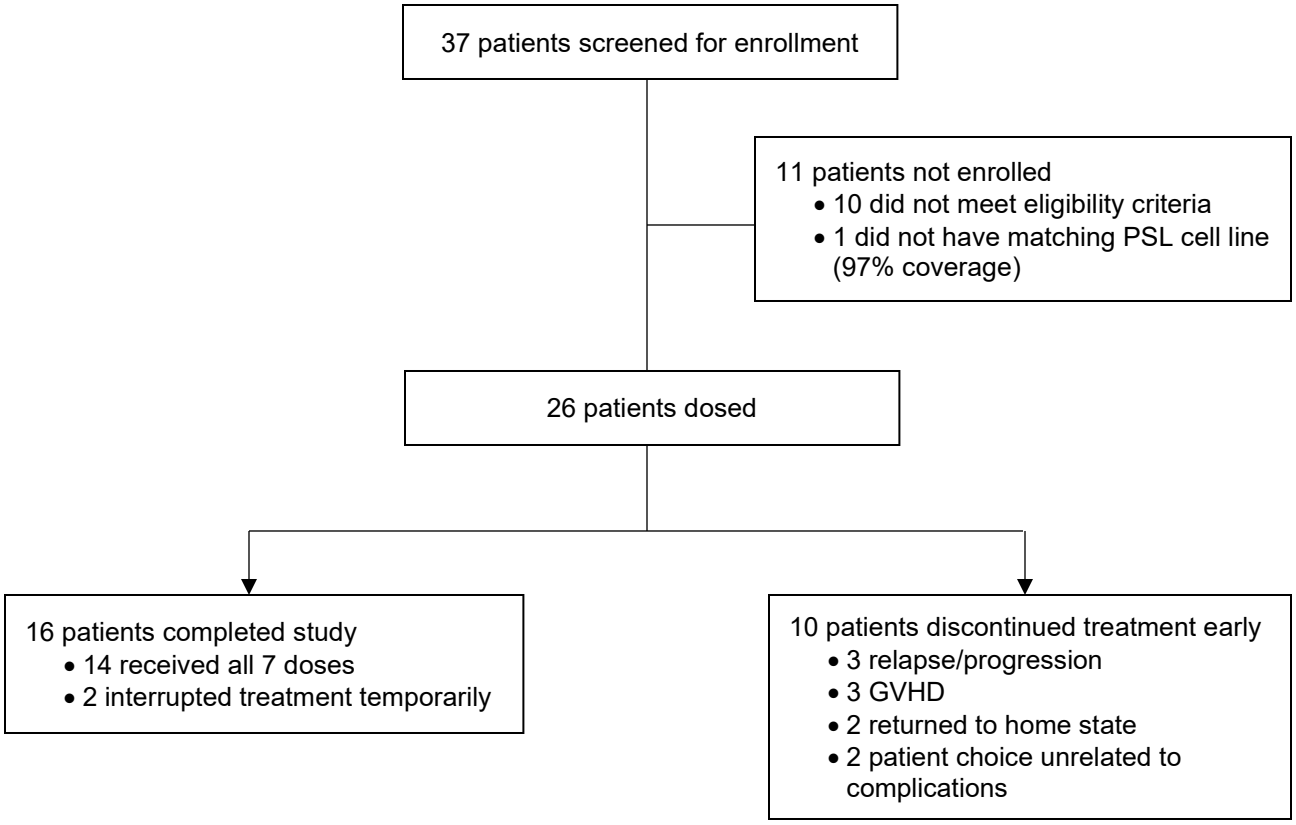
GVHD type	Age/Sex/ Disease	#PSL match, Txp Type	Source, Conditioning	Organ Class	Magic (max)	Outcome	Relatedness
Acute GVHD	61/F MDS	4/8 MMUD	PBSC, Reduced Intensity	GI	II	Recovered/ resolved	Not related
	60/F ALL	3/8 Haplo	PBSC, Myeloablative	Skin	II	Recovered/ resolved	Possibly
	44/M Adreno*	3/8 Cord + ATG	Cord, Myeloablative	Skin GI	III	Recovered/ resolved	Not related Not related
	69/M AML	3/8 MUD	PBSC, Reduced Intensity	Skin GI	IV	Recovered/ resolved	Not related Not related
	76/M AML	3/8 MMUD	PBSC, Reduced Intensity	GI	II	Recovered/ resolved	Not related

Supplementary Table 4. Cases of Treatment-Emergent Chronic GVHD

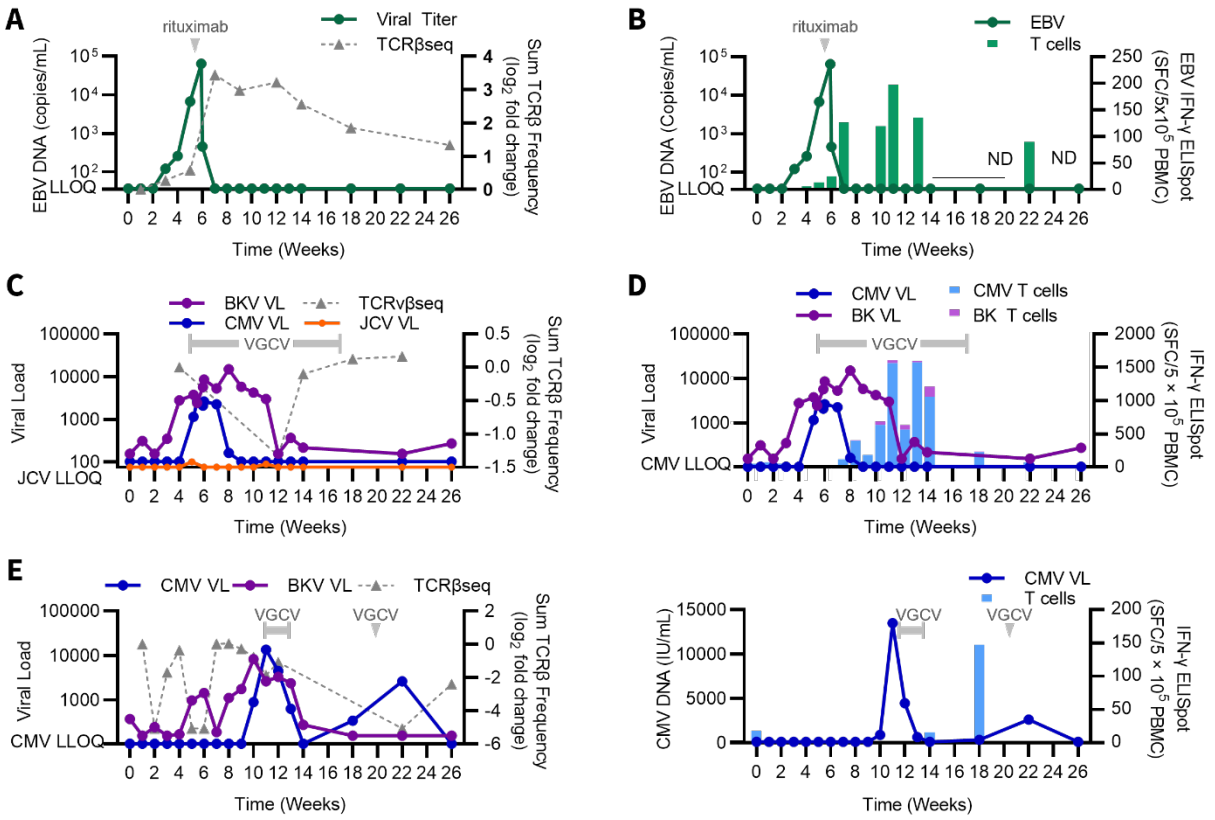
GVHD type	Age/Sex/ Disease	#PSL match, Txp Type	Source, Conditioning	Organ Class	Magic (max)	Outcome	Relatedness
Chronic GVHD	59/F AML	2/8 Haplo	Bone marrow, reduced intensity	Lung	Severe	Recovered/ resolved	Related
	73/F CML	3/8 Haplo	PBSC; reduced intensity	Skin	Severe	Not recovered/ not resolved	Not related
	44/M Adreno	3/8 Cord	Cord, Myeloablative	GI	--	Recovered/ resolved	Not related
	14/F AML	2/8 Haplo	Bone marrow, myeloablative	Liver	Severe	Not recovered/ not resolved	Not related
	76/M AML	2/8 MMUD	PBSC, reduced intensity	Oral	--	Recovered/ resolved	Related

*Adrenoleukodystrophy

Supplementary Figure 1. Patient Disposition



Supplementary Figure 2. Detection of Functional Immune Reconstitution and Posoleucel Clones Over Time in Patients with CSI During Primary Endpoint



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. Three patients with CSIs during the primary endpoint period (Week 14) are shown: patient #1: EBV CSI (A, B; received 5 doses of posoleucel, missed doses at Week 6 and 8); patient #2: CMV CSI and BK, JCV viremia (C, D; received all 7 doses of posoleucel), and patient #3: CMV CSI and BKV 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 10^5 PBMCs). In right panels showing ELISpot data, only viremia for which there was corresponding ELISpot data is shown. All detectable viremia (viremia > LLOQ) is shown in the left panels with TCRβ sequencing data.