Safety and Efficacy of Virus-Specific Cytotoxic T-Lymphocytes Manufactured by the IFN-γ Cytokine Capture System for the Treatment of Refractory Adenovirus, Cytomegalovirus, Epstein Barr Virus, and BK Virus Infections in Children, Adolescents and Young Adults after Allogeneic Hematopoietic Stem Cell Transplantation, Solid Organ Transplantation, or with Primary Immunodeficiency (IND# 17449)

Allyson Flower, MD[1,4,6], Rachel Friedmann, DO[6], Janet Ayello, MS, MT[ς6], Olivia Rigot, PhD[1], Lauren Harrison, RN, MSN[1], Erin Morris, RN[1], Meghan Sturhahn, RN, MSN[6], Elena Maryamchik, MD[7], Yongping Wang, MD, PHD[7], Lynn O'Donnell, PHD[8], Rolla Abu-Arja, MD[9], Dean Lee, MD, PHD[9], Julie Talano, MD[10], Bryon D. Johnson, PHD[10], Nancy Bunin, MD[7], Mitchell S. Cairo, MD[1,2,3,4,5,6]

Department of Pediatrics[1], Medicine[2], Pathology [3], Microbiology and Immunology[4], Cell Biology and Anatomy[5], New York Medical College, Valhalla, New York; Westchester Medical Center[6], Valhalla, New York; Department of Pediatrics[7], Children's Hospital of Philadelphia , Philadelphia, Pennsylvania; Department of Hematology[8], Ohio State University, Columbus, Ohio; Department of Pediatrics[9], Nationwide Children's Hospital, Columbus Ohio; Department of Pediatrics[10], Medical College of Wisconsin, Milwaukee, Wisconsin

Background: Viral infection remains a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT) (Bollard/Heslop *Blood* 2016). Anti-viral agents for treatment of viral infection in immunocompromised patients are limited in efficacy and are associated with significant toxicities (Gerdemann *BBMT* 2004; Sili *Cytother* 2012). The use of virus-specific cytotoxic T-lymphocytes (VST) for immunocompromised patients with viral infections has been associated with therapeutic benefit and improved OS (Bollard/Heslop *Blood* 2016; Sutrave *Cytother* 2017). Methods of VST production include ex-vivo expansion and direct selection (Gottlieb *Cytother* 2017). Ex-vivo expansion requires prolonged manufacturing time, is associated with T-cell exhaustion, and results in a limited donor pool. Direct selection is rapid (12-24 hours), can be done locally, allows for expanded HLA matching, permits a low degree of HLA match to the recipient, and can be adapted for many viruses. A multicenter consortium, the Viral Cytotoxic T-Lymphocyte Consortium (VIRCTLC) was created to investigate the safety and efficacy of VST manufactured by direct selection using the IFN-γ Cytokine Capture System process automated on the CliniMACS® Prodigy device (Miltenyi Biotec) for immunocompromised patients with viral infection **(Figure 1)**.

Objective: Determine the safety and efficacy of VST for the treatment of immunocompromised child, adolescent and young adult (CAYA) patients with refractory, systemic viral infection and/or viral infection and intolerance to appropriate anti-viral medical therapy.

Design/Methods: CAYA patients after allo-HSCT, solid organ transplantation (SOT), or with primary immunodeficiency (PID) with refractory adenovirus (ADV), cytomegalovirus (CMV), Epstein Barr virus (EBV) or BK virus (BKV) infections as evidenced by increasing serum RT-PCR DNA (by 1 log) after 7 days or persistent quantitative RT-PCR DNA copies after 14 days of appropriate anti-viral therapy, and/or known resistance to anti-viral agents, and/or intolerance to anti-viral agents were eligible. Related donors with ≥1 HLA A, B, or DR match to recipient and with an adequate T-cell response to virus specific MACS® PepTivators were eligible. Donors were screened with viral specific antigen (PepTivator®) to predict successful VST

manufacturing. Peripheral blood mononuclear cells (PBMC) were collected from eligible related donors using non-mobilized apheresis. VST were isolated using the CliniMACS® Prodigy following stimulation of PBMC with specific viral MACS PepTivator® pools, generously provided by Miltenyi Biotec. Production of CD4+ and CD8+ VST was performed as previously described (Feuchtinger *Blood* 2010). The target cell dose was $0.5x10^4$ $CD3^+$/kg for HLA mismatched haploidentical related donors and $2.5x10^4$ $CD3^+$/kg for matched related donors. Based on response and safety, VST were given every 2 weeks for a maximum of 5 infusions.

Results: Eleven patients have been enrolled to date. Seven patients were treated for ADV, 2 for BKV, 1 for CMV, and 1 for EBV. There were 8 males and 3 females enrolled, aged 1-38 years. There were 10 patients post allo-HSCT and 1 patient post SOT. There were 8 haploidentical, related, original allo-HSCT donors and 3 haploidentical, related, third party donors. There have been no matched related donors enrolled to date. The mean±SEM %CD4+ IFN-γ+ of total CD4+, %CD8+ IFN-γ+ of total CD8+, and %CD3 cells recovered in the final product were 21.5±4.8, 25.0±7.0, and 50.4.2±7.2, respectively. The median number of VST infusions was 2 (1-5). The mean±SEM $CD3^+$ cell dose was $0.49±0.001x10^4$. Ten patients achieved complete response (PCR negative) and 1 patient achieved partial response (PCR≥1 log decrease). The overall response and complete response rates were 100% and 90.9%, respectively. The median time to maximal response was 34 days (7-141) **(Table 1)**. No patient developed aGVHD, cGVHD, infusion reaction or CRS associated with VST.

Conclusion: Preliminary results of this pilot study demonstrate that VST are safe, well tolerated and efficacious in CAYA with refractory viral infections after allo-HSCT, SOT or with PID. Manufacturing utilizing the CliniMACS® Prodigy device is rapid, reproducible and effective. Accrual is ongoing. This research is supported by FDA RO10063-01A1.

Figure 1

CMV, ADV, EBV and BK Cytotoxic T-Lymphocytes (CTLS) Consortium (VIRCTLC) for Refractory EBV, CMV and ADV Viral Infections

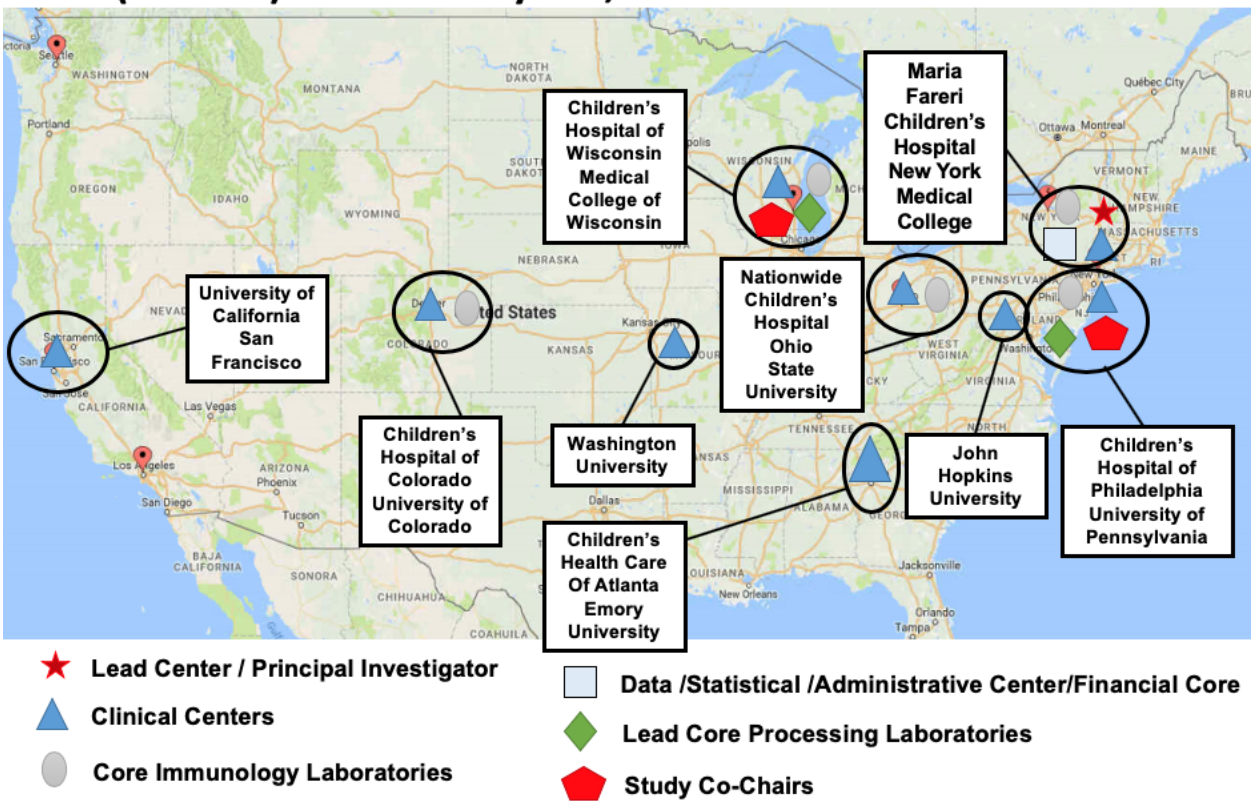

Table 1

Viral CTL Patient Characteristics and Response							
Age (Years)	**Virus**	**%CD4+IFN-γ+ of CD4+**	**%CD8+IFN-γ+ of CD8+**	**%CD3 Recovered**	**# of Infusions**	**Response**	**Time to Best Response (Days)**
11	ADV	46.5	42.1	56.0	3	CR	53
		33.0	20.6	28.0			
10	ADV	57.0	53.2		1	CR	7
1.5	ADV	21.4	8.8	68.0	5	CR	58
13	ADV	31.0	7.8		1	CR	34
38	ADV	10.3	6.2	62.0	2	CR	14
1	ADV	17.1	3.5	53.6	4	CR	14
16	ADV	3.8	38.0		1	CR	22
20	CMV	12.4	52.8	61.0	2	CR	47
8	BK	1.8	4.2	78.0	5	CR	141
		21.9	15.0	43.0			
		0.6	4.6	10.0			
17	BK	26.7	4.0	30.0	3	CR	44
		25.0	39.0	65.0			
30	EBV	14.1	75.2		2	PR	7
Median 12		Mean±SEM 21.5±4.8	Mean±SEM 25.0±7.0	Mean±SEM 50.4.2±7.2	Median 2	CR - 10 PR - 1	Median 34