Safety and Efficacy of GMP Clinical Grade Manufacturing of Virus Specific Cytotoxic T Lymphocytes (vCTLS) against Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Adenovirus (ADV) and BKV to Treat Medically Refractory Viral Infections Post Allogeneic Hematopoietic Stem Cell Transplantation (AlloHSCT) in Children, Adolescents and Young Adults (CAYA) (IND# 17449)

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Severe and fatal viral infections, including EBV, CMV, ADV and BK virus, remain a major cause of morbidity and mortality in AlloHSCT recipients (Sili et al, *Cytotherapy*, 2012). While pharmacological agents are standard therapy for some viral infections, most have substantial toxicities, are frequently ineffective and development of resistant strains is a problem (Erdmann et al. *Molecular Therapy*, 2012; Sili et al. *Cytotherapy* 2012.). We report a GMP process that rapidly generates within 24 hrs., (CD4+ and CD8+) vCTLs that is consistently specific for antigens derived from the viruses EBV, CMV, ADV or BKV.

OBJECTIVE: The aim of this study is to determine the safety and efficacy of vCTLS manufactured from haploidentical stem cell donors with specificity for CMV, ADV, EBV or BKV post AlloHSCT setting.

DESIGN/METHODS: This is a phase II multicenter, multidisciplinary FDA approved clinical trial (Figure 1). Eligibility included 7 pediatric patients with medically refractory viral infections (ADV, CMV, EBV or BKV). Haploidentical donors were screened to the specific viral peptivator using the IFN γ cytokine secretion assay. If donor response was >0.01%IFNγ, a short apheresis of MNCS were collected by the Spectra OPTIA ® apheresis instrument. The virus specific CTLs were manufactured using the CliniMACS ® Prodigy, IFN γ Cytokine Capture System (CCS) with incubation of MACS GMP PepTivator® peptide pools. Identification of T cell subsets (CD3,CD4,CD8), IFNγ and cell viability were determined by flow cytometry. vCTLS were administered as either fresh infusion or cryopreserved products that were thawed. vCTLS were cryopreserved in 10% DMSO using a rate-controlled freezer and stored in LN_2 vapor phase. Cell doses were limited to 0.5x104 CD3/kg in HLA mismatched related donors. Additional doses of vCTLs were given every 2 weeks, for a maximum of 5 infusions.

RESULTS: Seven patients, ages 1 yr. to 38 yrs., received vCTLs on this study: CMV (1), ADV (4) and BKV (2). The mean±SEM CD3 cell dose infused was 0.5±0.001 x10^4/kg . The mean ±SEM % CD4/IFNγ, %CD8/IFNγ and % total IFNγ$^+$ cells in the final vCTL product were 24±4.7%, 20.5±7.5%, 40±8.7%, respectively. Viability of CD3 cell dose infused was ≥ 70%, endotoxin negative and gram stain negative. There have been no episodes of ≥grade II-IV AGVD, chronic GVHD, CRS or infusion related reactions due to the vCTL infusions. One patient is too early to evaluate. Among the 6 other patients, there have been 6 CRs .(Table 1)

The preliminary results demonstrate that vCTLS can be manufactured in a reproducible, standardized, safe and efficient manner, well tolerated and efficacious in CAYA with refractory viral infections post AlloHSCT. Accrual is ongoing. The research is supported by FDA RO10063-01A1.

FIGURE 1

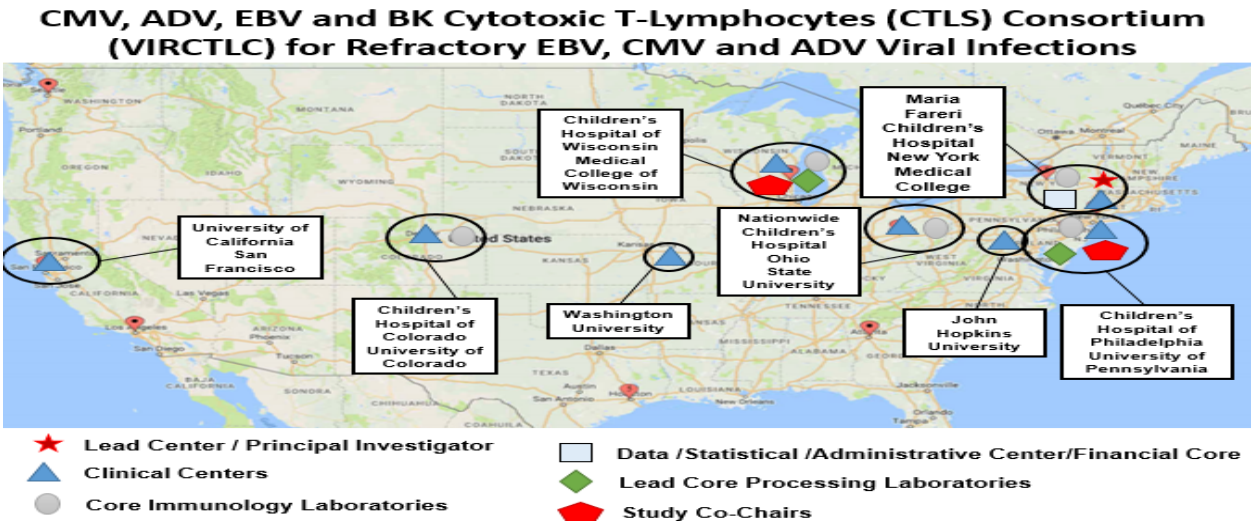

TABLE 1

AGE (Years)	VIRUS	M/F	CTL Donor HLA Match	Diagnosis	# of Infusions	Cell dose x10^4/kg	%CD4/IFNγ *	%CD8/IFNγ*	Total % IFNγ*	Response	Infusion Reaction or CRS	Time to Best Response (Days)	aGVHD 2° to vCTLs
11	ADV	M	8/10	AML	3	0.5	39.5	31.35	42	CR	NONE	53	NONE
1.5	ADV	F	5/10	SCD	5	0.5	21.4	8.8	30.2	CR	NONE	58	NONE
38	ADV	M	8/10	ALL	2	0.5	10.3	6.2	16.5	CR	NONE	14	NONE
1	ADV	M	5/10	SCD	4	0.5	17.1	3.5	20.6	CR	NONE	14	NONE
20	CMV	M	5/10	SCD	2	0.5	12.4	52.8	65.2	CR	NONE	47	NONE
8	BKV	M	5/10	SCD	5	0.5	44.1	19.4	63.5	***	NONE	Still evaluating	NONE
17	BKV	F	5/10	AML	5	0.5	25.68	25	51.68	CR	NONE	44	NONE
MEAN±SEM							24.1±4.7	20.5±7.5	40.0±8.7				

* Mean of infusions
*** (still evaluating)