SUPPLEMENTAL MATERIAL

IND-enabling studies for a clinical trial to genetically program a persistent cancer-targeted immune system

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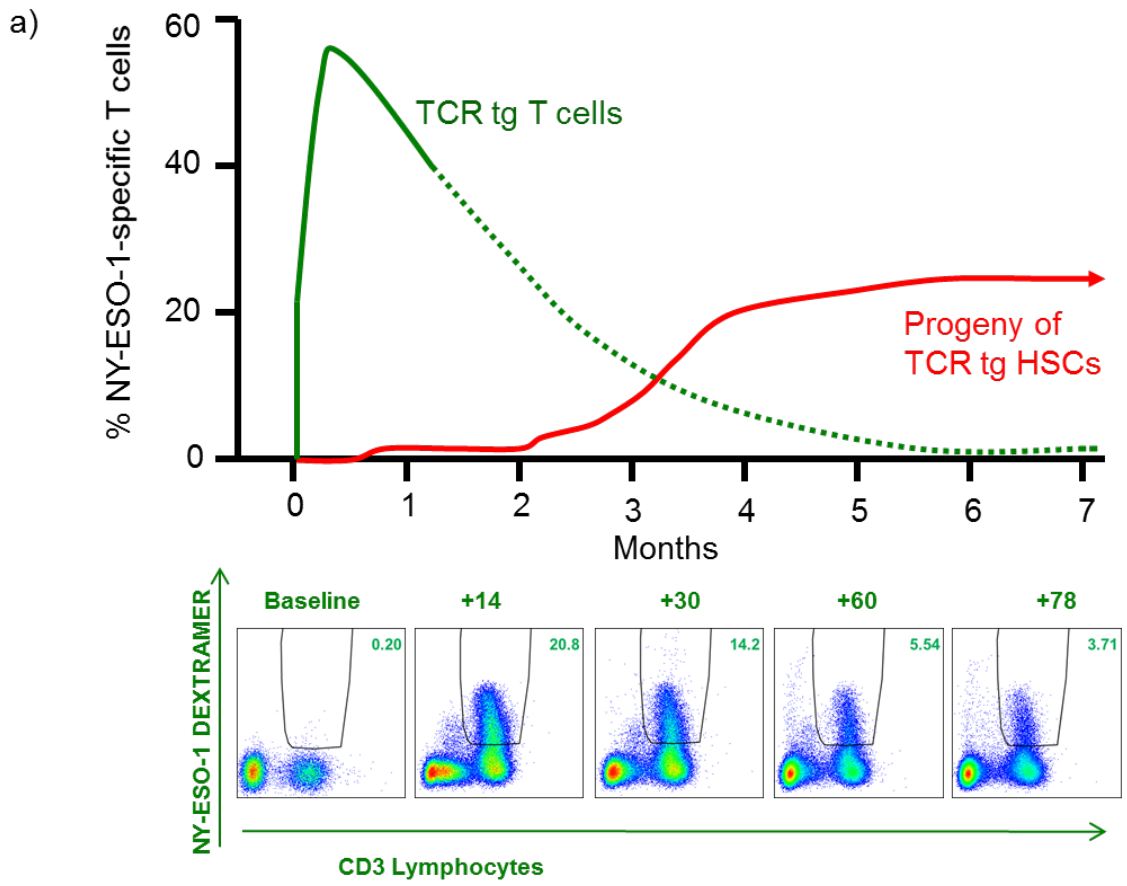
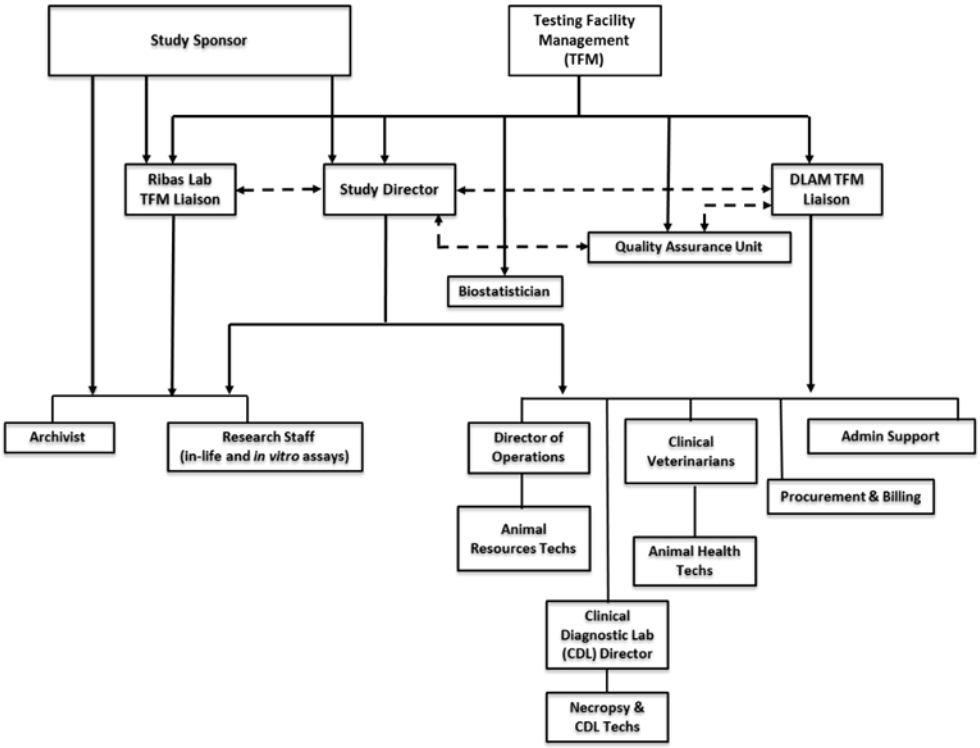
Included in these supplemental materials are:

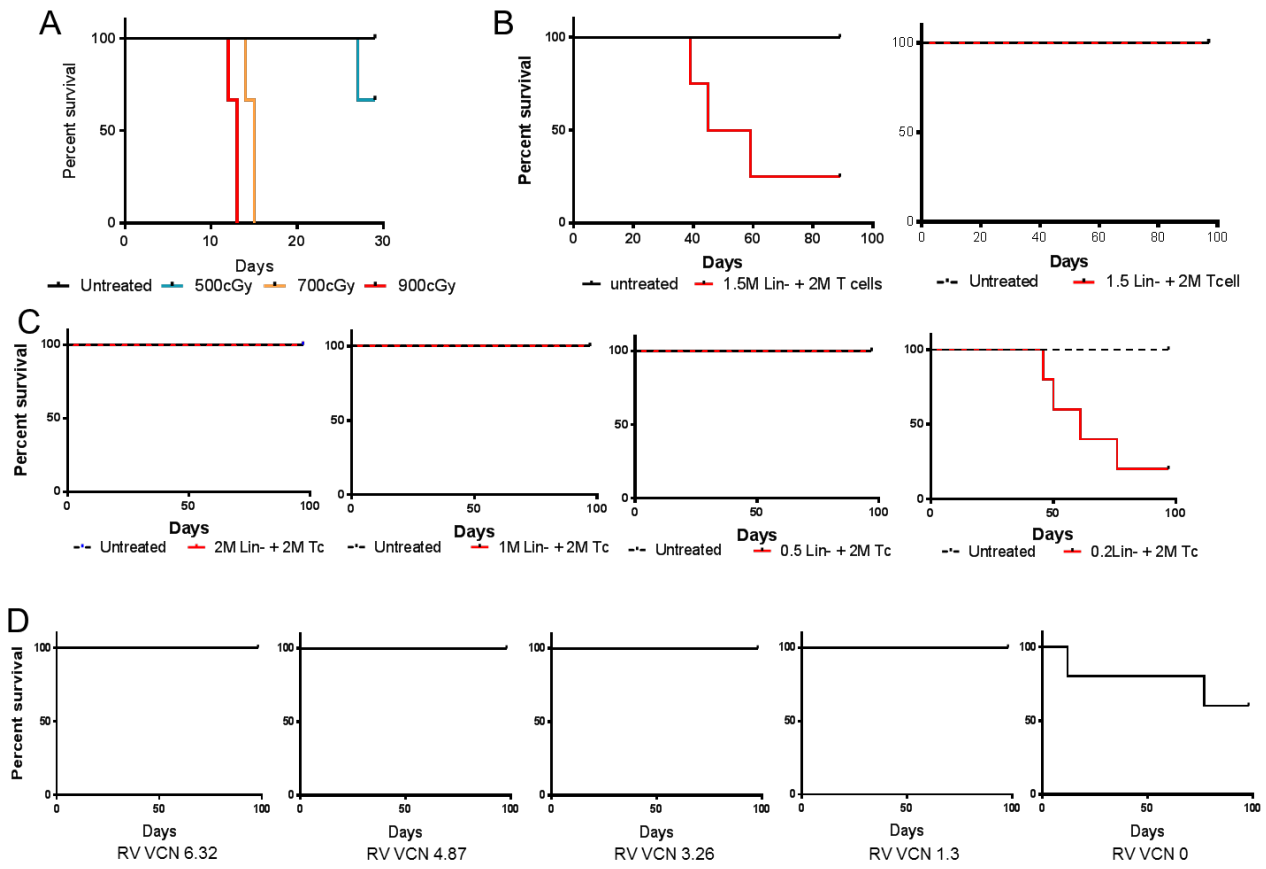


Supplemental Figure 1: Hypothetical model of peripheral blood TCR-transgenic cell repopulation. A. Green line: Peripheral blood levels of TCR-transgenic cells as a result of the adoptive cell transfer of mature lymphocytes expressing NY-ESO-1 TCR. These cells expand briskly over the first two weeks and achieve high peripheral blood levels, followed by a more protracted contraction. Dotted green line: Our functional studies demonstrate that these cells lose their initial high lytic activity and become immune deviated (shift in cytokine profile upon antigen exposure) but still remain antigen-specific effector cells. Red line: TCR-transgenic cells as a result of the endogenous re-population of peripheral tissues by TCR-transgenic HSCs will first appear in the periphery by 1-3 months and will provide a continuous source of TCR-transgenic cells with maintained antitumor functionality. **B.** Representative analysis of TCR-transgenic T cells by MHC tetramer assay in blood from a patient enrolled in the mature TCR-transgenic ACT clinical trial. The initial expansion of TCR transgenic cells is followed by a decrease in their frequency over time.

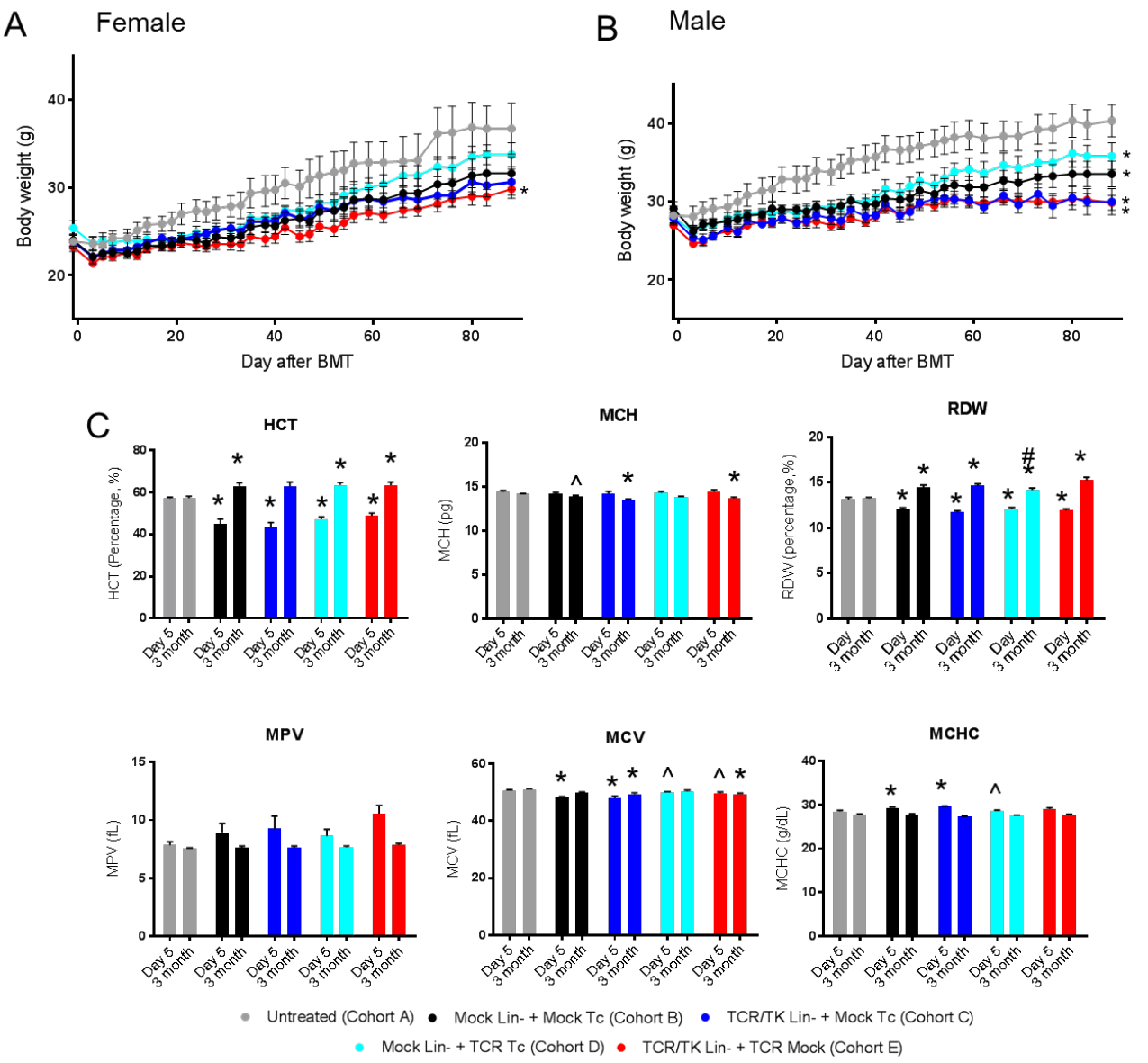


Supplemental Figure 2: GLP team organizational chart. Due to the complexity of this GLP study, no single team could provide personnel with all of the specialized skills required. The study was undertaken as a collaborative effort among members of the Ribas laboratory and members of the Department of Laboratory Animal Medicine (DLAM) in UCLA. A total of 32 staff were involved in the study. The sponsor initiated the study, financially supported it and submitted the IND application to the FDA. Test Facility Management (TFM) Liaisons were appointed to oversee the facilities and the personnel of each group and facilitate communication with the Study Director and the TFM. The Study Director was the central point of control of the study and had overall responsibility for the technical conduct of the study, the interpretation, analysis, documentation and reporting of results. An independent Quality Assurance Unit inspected the facility and the procedures to ensure GLP regulations were followed and also reviewed all documentation and reports generated.

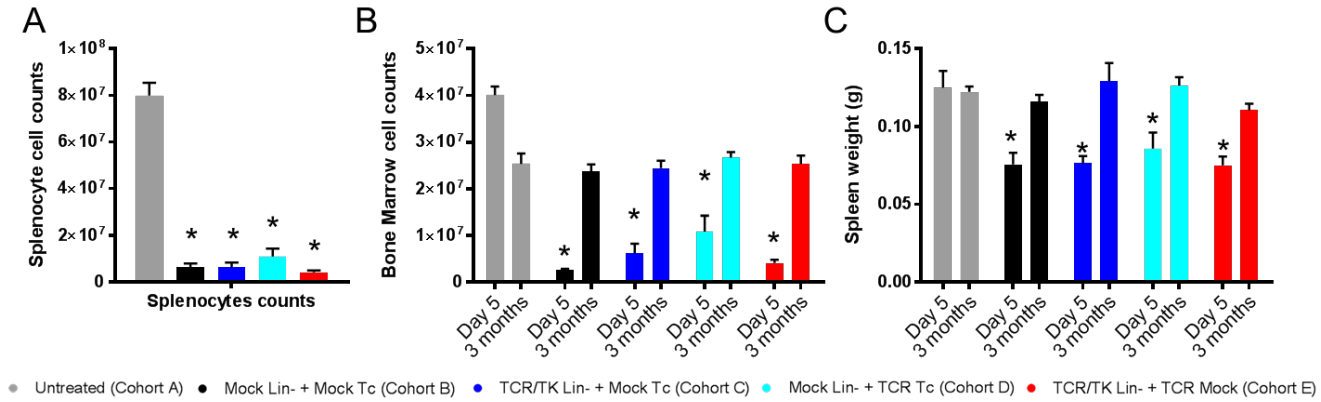
The solid line indicates personnel reporting structure for this study. The dotted line indicates lines of communication.



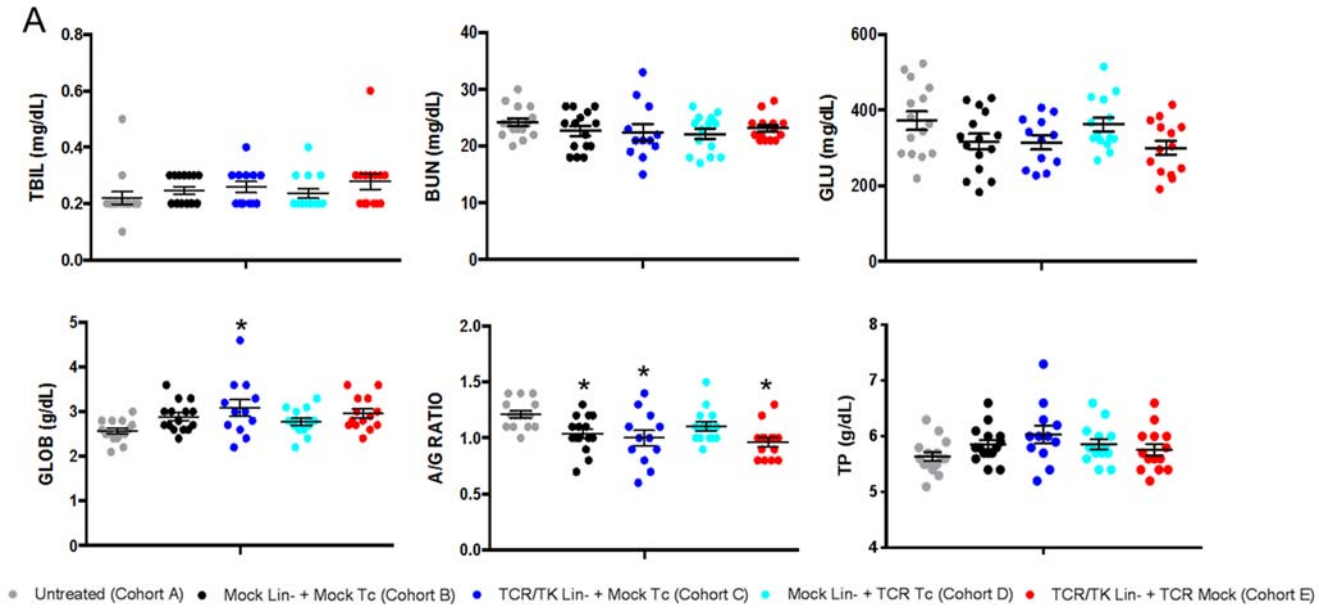
Supplemental Figure 3. Bone marrow transplant (BMT) optimization studies in HLA-A2/K^b transgenic mice. A. Mice were untreated or received an irradiation dose of 500, 700 or 900cGy respectively. Mice were followed for 30 days. Kaplan-Meier survival curves after irradiation (n= 3-6). **B.** Lin- cells were purified from the bone marrow of HLA-A2/K^b donor mice, and either prestimulated for 18-20h, transduced with the LV-NY-ESO-1 TCR/sr39TK and cultured for 24h more (short culture) or prestimulated for 48 hours, transduced with the same vector and cultured for 48 hours more (long culture). T cells were purified from the spleen of HLA-A2/K^b donor mice, activated for 16h, transduced with RV-NY-ESO-1 TCR and expanded for 3 days. 1.5million Lin- cells and 2 million T cells were co-administered to HLA-A2/K^b mice that had previously received TBI (900cGy). Mice were followed for 3 months. Kaplan-Meier survival curve after BMT with long culture Lin- cells (left) and short culture Lin- cells (right), (n=4-5). **C.** Short culture Lin- cell dose escalation. Two, 1.5, 1.0, 0.5 or 0.2 million short culture Lin- cells transduced with the LV-NY-ESO-1 TCR/sr39TK were co-administered with 2 million T cells transduced with the RV-NY-ESO-1 TCR to myelodepleted HLA-A2/K^b mice. Mice were followed for 3 months. Kaplan-Meier survival curve. Data for the 1.5 million Lin- cohort is in figure S3B (right), (n=4-5). **D.** T cell VCN dose escalation. 1.5 million short culture Lin- cells transduced with the LV-NY-ESO-1 TCR/sr39TK were co-administered with 2 million T cells transduced with decreasing amounts of the RV-NY-ESO-1 TCR to myelodepleted HLA-A2/K^b mice. The retrovirus VCN in the T cells is indicated in the figure. Mice were followed for 3 months. Kaplan-Meier survival curve, (n=5).



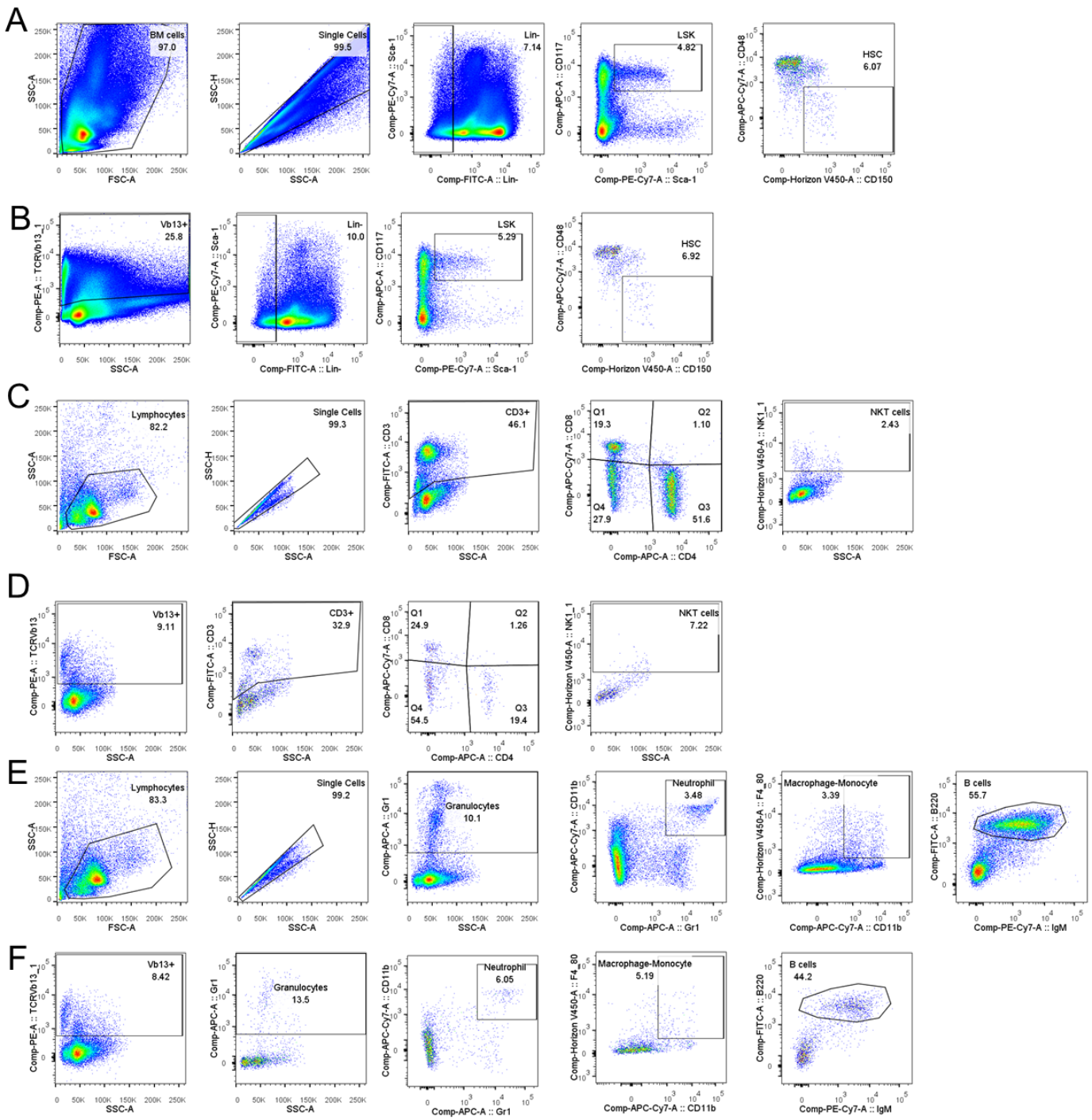
Supplemental Figure 4. Body weight and hematology assessment at day 5 and 3 months after BMT. TCR-engineered Lin- cells and T cells were co-administered in myelodepleted HLA-A2/K^b mice. Mice were followed for three months after BMT, and total body weight was measured 2-3 times a week. Body weight was divided by gender. **A.** Female (n=8). **B.** Male (n=8). * p<0.05 vs cohort A pair-wise comparisons of least-squares means in a linear model framework with Tukey-Kramer adjustment within each time point, considered significant only if 5 or more consecutive measurements were significant. **C.** Hematology at 5 days (n=6) and 3 months (n=12-15) after BMT. HCT, hematocrit; MCH, mean corpuscular hemoglobin; RDW, red cell distribution width; MPV, mean platelet volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration. Mean ±SEM is plotted. * p<0.05 vs cohort A, ^ p<0.05 vs cohort C, # p<0.05 vs Cohort E, pair-wise comparisons of least-squares means in a linear model framework with Tukey-Kramer adjustment within each time point.



Supplemental Figure 5. Spleen and bone marrow cellularity at day 5 and 3 months after BMT. TCR-engineered Lin- cells and T cells were co-administered in myelodepleted HLA-A2/K^b mice. Mice were followed for three months after BMT. Mice from each cohort were euthanized at day 5 (n=6) and 3 months (n=12-15) after BMT. **A.** Splenocyte counts at 5 days. **B.** Bone marrow cell counts at 5 days and 3 months after BMT. **C.** Spleen weight at 5 days and 3 months after BMT. Mean ±SEM is plotted. * $p<0.05$ vs cohort A, pair-wise comparisons of least-squares means in a linear model framework with Tukey-Kramer adjustment within each time point.

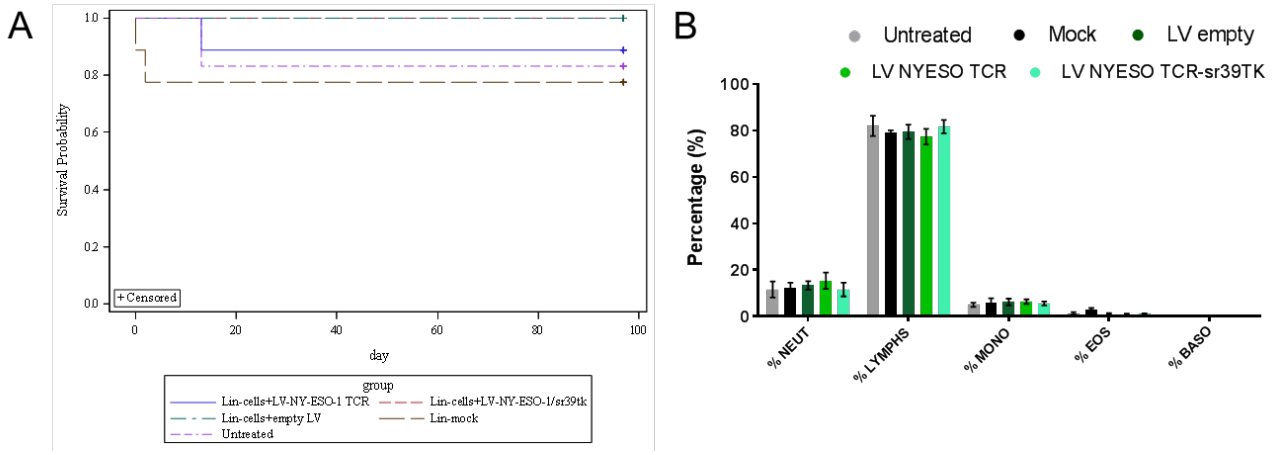


Supplemental Figure 6. Serum chemistry at 3 months after BMT. TCR-engineered Lin- cells and T cells were co-administered in myelodepleted HLA-A2/K^b mice. **A.** Mice were followed for 3 months after BMT (n=12-15). TBIL, total bilirubin; BUN, blood urea nitrogen; Gluc, glucose; Glob, globulin; A/G, albumin to globulin ratio; TP, total protein. * $p<0.05$ vs cohort A, pair-wise comparisons of least-squares means in a linear model framework with Tukey-Kramer adjustment.

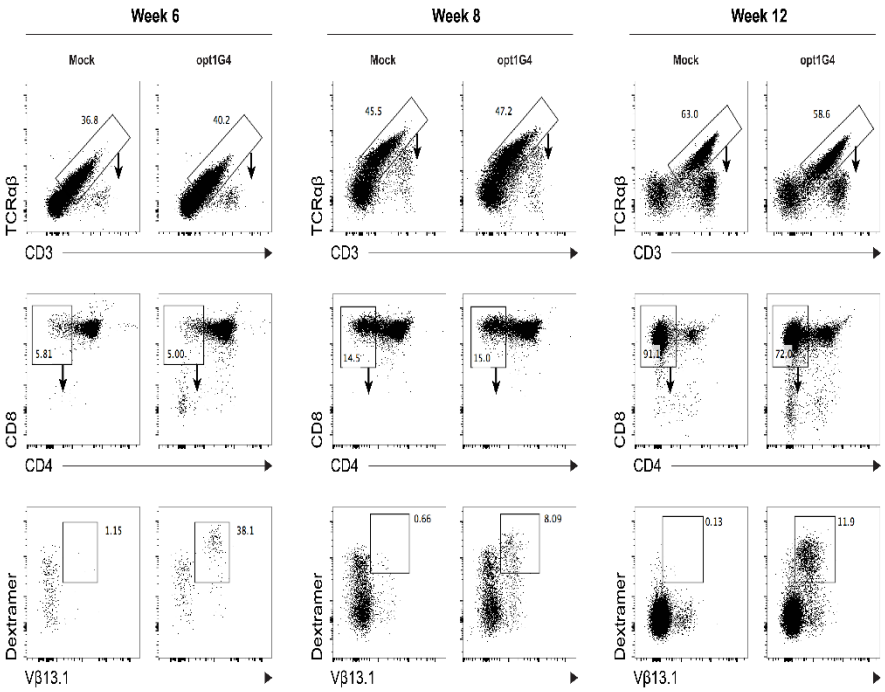


Supplemental Figure 7. Flow cytometry gating strategy for bone marrow and splenocytes phenotype characterization. TCR-engineered Lin- cells and T cells were co-administered in myelodepleted HLA-A2/K^{b} mice. At 3 months after BMT phenotype characterization of the bone marrow cells and the splenocytes was performed. Gating strategy is shown. **A.** The lineage-negative (Lin-) cell population was gated on the total bone marrow single cells. LSK population was measured

by gating the $Scal^+$ $c\text{-}kit^+$ cells in the Lin^- population. The hematopoietic stem cell population (HSC) was gated on the $CD48^-$ $CD150^+$ LSK population. **B**. The Lin^-, LSK and HSC populations were also measured in the $V_\beta13^+$ bone marrow single cells. **C**. The $CD3^+$ population was gated on the splenocyte single cells population. The $CD4^+$, $CD8^+$ and NKT ($CD3^+$ $NK1.1^+$) cells were gated on the $CD3^+$ population. **D**. Same gating strategy as in C was applied to the total $V_\beta13^+$ splenocyte single cell population. **E**. Granulocytes ($Gr1^+$), neutrophils (Gr^+, $CD11b^{hi}$), macrophages-monocytes ($CD11b^+$ $F4\text{-}80^+$) and B cells (IgM^+ $B220^+$) were gated on the splenocyte single cell population. **F**. The same gates as in E were applied to the $V_\beta13^+$ splenocyte single cell population.



Supplemental Figure 8. Survival and hematology 3 months after BMT with Lin- cells transduced with LV-empty, LV-NY-ESO-1 TCR or LV-NY-ESO-1 TCR/sr39TK. TCR-engineered Lin- cells transduced with LV-NYESO-1 TCR/sr39TK, LV-NYESO-1 TCR or empty vector were transplanted in myelodepleted HLA-A2/K^{b} mice. Mice were followed for 3 months after BMT. **A.** Kaplan-Meier survival curve. No significant differences by Log-rank test comparing median survival among the different treatment groups (p=0.3858, n=6-9). **B.** White Blood Cell differential at 3 months after BMT in percentages (n=5-9). Neut, neutrophils; Lymphs, lymphocytes; Mono, monocytes; Eos, eosinophils; Baso, basophils. No significant differences were observed by Pair-wise Comparison with Tukey-Kramer adjustment.



Supplemental Figure 9. Differentiation of NYESO TCR/sr39TK-engineered T cells in ATOs (artificial thymic organoids). Fresh CD34+ cells were enriched using CliniMACS. CD34+CD3- cells were purified by FACS sorting. CD34+CD3-were transduced with LV-NYESO TCR/sr39TK or mock-transduced (no virus added) and cultured in ATOs for the indicated times. Analysis shows total ATO cells (top row) gated on DAPI-CD14-CD56-mCD29- cells (to gate out dead cells, monocytes/macrophages, NK cells, and murine stromal cells, respectively). Sequential gates are shown by arrows. CD3+TCRab+CD8SP T cells expressing NY-ESO-1 TCR (1G4 clone) are indicated by the $V_{\beta}13$+ dextramer+ gate.

Supplemental Table 1. Certificate of Analysis of the GMP-comparable LV-NY-ESO-1 TCR/sr39TK (RRL-MSCV-optNYESO-optsr39TK-WPRE, production volume: 20L).

TEST	METHOD	ACCEPTABILITY CRITERIA	RESULTS
Physical viral vector titer	P24 ELISA	Report results	3.9×10^7 pg/mL
Infectious titer	Assay using HT29 cells	Report results	6.8×10^8 ifu/mL
Sterility	Aerobic and anaerobic culture for bacteria and fungus	No growth within 14 days	No growth within 14 days
Mycoplasma	Culture and Vero indicator cells	Negative	Negative
Endotoxin	Limulus amebocyte lysate	<100 EU/mL per sample	<0.06 EU/mL

EU, endotoxin units; Ifu, infectious units

Supplemental Table 2. Certificate of Analysis of the Clinical Grade Lentivirus LV-NY-ESO-1 TCR/sr39TK (RRL-MSCV-optNYESO-optsr39TK-WPRE, production volume 60L).

TEST	METHOD	ACCEPTABILITY CRITERIA	RESULTS
Vector insert	Southern Blot analysis	Vector size consistent with predicted fragment size	Vector size consistent with predicted fragment size
Physical viral vector titer	P24 ELISA	≥ 2.5×10^7 pg/mL	4.0×10^7 pg/mL
Infectious titer	Assay using HT29 cells	≥ 2.0×10^7 ifu/mL	2.0×10^8 ifu/mL
Sterility	Aerobic and anaerobic culture for bacterial and fungal contamination	No growth within 14 days	No growth within 14 days
Mycoplasma contamination	Culture and Vero indicator cells	Negative	Negative
In vitro viral assay	Assay on MRC-5, Vero and A549 cells	No CPE or hemadsorption	No CPE or hemadsorption
Replication competent lentivirus testing (RCL)	Co-culture of end production cells with C8166 cells with amplification and indicator phases. Supernatant testing on C8166 cells with amplification and indicator phases.	No evidence of RCL	No evidence of RCL
Endotoxin concentration	Limulus amebocyte lysate	<100 EU/ml each sample	>30 and < 48 EU/ml
Residual total viral DNA	Quantitative PCR (qPCR)	Report result	9.14×10^5 fg/µL
Residual benzonase	ELISA	Report result	Below limit of detection
Transfer of residual E1A	qPCR	Negative	<10 copies/0.2µg DNA[a]
Transfer of residual SV40	qPCR	Negative	<10 copies/0.2µg DNA[a]

[a]The limit of detection for this assay is 10 copies/0.2µg DNA. A result of <10 copies/0.2 µg DNA is considered negative.

EU, endotoxin units; ifu, infectious units; CPE, cytopathic effect; RCL, replication-competent lentivirus

Supplemental Table 3. Certificate of Analysis of the clinical grade RV-NY-ESO-1 TCR (MSGV1-A2aB-1G4A-LY3H10) (Production volume 18L)*.

TEST	METHOD	LIMITS	RESULTS
Sterility	Aerobic and anaerobic culture for bacterial and fungal contaminants	No growth 14 days	No growth 14 days
Mycoplasma contamination	Mycoplasma culture	Negative for presence of Mycoplasma	Negative for presence of Mycoplasma
Adventitious virus contamination	In-vitro viral assay utilizing 3T3, MRC-5 and Vero cells	No CPE or hemadsorption	No CPE or hemadsorption
Endotoxin concentration	Limulus amebocyte lysate	< 0.33 EU/mL	Harvest 1-6: less than 0.06 EU/mL
Replication competent retrovirus: GAL-V	S+L-(PG-4) (293 infection) 5% of vector supernatant	No evidence of RCR	No evidence of RCR
	S+L-(PG-4) (293 co-culture) 10^8 cells from production run	No evidence of RCR	No evidence of RCR
Transgene expression	NY-ESO-$1_{157-165}$ tetramer or dextramer staining in activated PBMC	> 10% NY-ESO-$1_{157-165}$ tetramer or dextramer positive cells among CD3+ T lymphocytes	41.5% NY-ESO-$1_{157-165}$ dextramer positive cells among CD3+ T lymphocytes
Potency	NY-ESO-$1_{157-165}$ peptide and antigen-specific IFN-γ production by ELISA	> 30,000 pg/ml of IFN-γ production upon NY-ESO-$1_{157-165}$ peptide stimulation using NY-ESO-$1_{157-165}$ peptide-pulsed K562/A2.1 cells	> 300,000 pg/ml of IFN- γ production upon NY-ESO-$1_{157-165}$ peptide stimulation using NY-ESO-$1_{157-165}$ peptide-pulsed K562/A2.1 cells

CPE, cytopathic effect; EU, endotoxin units; PBMCs, peripheral blood mononuclear cells; RCR, replication-competent retrovirus

*Viral vector produced using a PG13 master cell bank (PG13-A2aB-1G4A-LY-3H10 MCB) obtained from Dr. Rosenberg. A cross-reference letter was obtained from Dr. Rosenberg in support of the IND for this clinical trial.

Supplemental Table 4. Certificate of Analysis of the GMP comparable RV-NY-ESO-1 TCR (MSGV1-A2ab-1G4A-Ly3H10, Production volume: 3L).

TEST	METHOD	ACCEPTABILITY CRITERIA	RESULTS
Sterility	Aerobic and anaerobic culture for bacterial and fungal contaminants	No growth within 14 days	No growth within 14 days
Mycoplasma contamination	qPCR	Negative	Negative
Endotoxin	Limulus amebocyte lysate	<100 EU/mL	<0.06 EU/mL

EU, endotoxin units

Supplemental Table 5. Co-Administration of NY-ESO-1 TCR Genetically Modified T cells and Hematopoietic Stem Cells (HSCs) in HLA-A2.1/K[b] mice. GLP studies cohort distribution.

Group	XRT (cGy)	Treatment		5 days post-BMT	3 Month post-BMT[b]
		T cells	Lin- cells		
Cohort A	No	No	No	3M/3F	8M/8F
Cohort B	900	2x 10^6 Mock-Transduced T cells	1.2-1.3 x 10^6 Mock-Transduced Lin- cells[a]	3M/3F	8M/8F
Cohort C	900	2 x 10^6 Mock-Transduced T cells	1.2-1.3 x 10^6 TCR-Transduced Lin- cells[a]	3M/3F	8M/8F
Cohort D	900	2 x 10^6 TCR-Transduced T cells	1.2-1.3 x 10^6 Mock-Transduced Lin- cells[a]	3M/3F	8M/8F
Cohort E	900	2 x 10^6 TCR-Transduced T cells	1.2-1.3 x10^6 TCR-Transduced Lin- cells[a]	3M/3F	8M/8F

[a] The study was divided into 3 experiments. 1.2 x10^6 Lin- cells were injected for Experiment 2 and 1.3 x10^6 Lin- cells were injected for Experiments 1 and 3. Animals in Experiments 1 and 2 were euthanized at 3 months after BMT, animals in Experiment 3 were euthanized at day 5 after BMT.

[b] Mice were sacrificed on two consecutive days (days 87 and 88 after BMT) to accommodate scheduling with external test sites and allow enough time to process and ship all samples. Mice from Cohort A were not irradiated.

XRT, Irradiation; BMT, bone marrow transplantation; Lin- cells, Lineage negative; M, Male; F, Female.

Supplemental Table 6. Cell manufacturing acceptance criteria.

Test Item		Experiment 1	Experiment 2	Experiment 3	Acceptability Criteria
T cell purity[a]		93.0%	95.0%	90.0%	>75%
Lin- purity:	Lin- frequency[b]	Batch 1: 45.2%[e] Batch 2: 45.5%[e] Batch 3: 52%[e]	Batch 1: 44.4% Batch 2: 48.6% Batch 3: 46.0%	Batch 1: 44.1% Batch 2: 38.4% Batch 3: 52.7%	>25%
	LSK frequency[c]	Batch 1: NA[e] Batch 2: NA[e] Batch 3: NA[e]	Batch 1: 2.22% Batch 2: 2.47% Batch 3: 2.35%	Batch 1: 3.13% Batch 2: 2.62% Batch 3: 2.9%	No limit established
LV VCN in Lin- cells (2 weeks after culture)		Mock: 0 TCR: 1.44	Mock: 0 TCR: 1.81	Mock: 0 TCR: 1.61	0.6-3
RV VCN in T cells (2 weeks after culture)		Mock: 0 TCR: 3.67	Mock: 0 TCR: 5.28	Mock: 0 TCR: 4.9	No limit established
T cell viability (day of administration)		Mock: 70.8% TCR: 66.7%	Mock: 88.6% TCR: 86.9%	Mock: 81.0% TCR: 77.6%	> 65%
Lin- cell viability (day of administration)		Mock: 69.3% TCR: 65.3%	Mock: 75.6% TCR: 69.2%	Mock: 68.4% TCR: 65.9%	> 65%
Mycoplasma contamination	T cells mock	Negative	Negative	Negative	Negative
	T cell transduced	Negative	Negative	Negative	Negative
	Lin- mock	Negative	Negative	Negative	Negative
	Lin- transduced	Negative	Negative	Negative	Negative
Endotoxin concentration	Cohort B:	<5EU/Kg	<5EU/Kg	<5EU/Kg	<5EU/Kg
	Cohort C:	<5EU/Kg	<5EU/Kg	<5EU/Kg	<5EU/Kg
	Cohort D:	<5EU/Kg	<5EU/Kg	<5EU/Kg	<5EU/Kg
	Cohort E:	<5EU/Kg	<5EU/Kg	<5EU/Kg	<5EU/Kg
Sterility[d]	T cells mock	Negative	Negative	Negative	Negative
	T cell transduced	Negative	Negative	Negative	Negative
	Lin- mock	Negative	Negative	Negative	Negative
	Lin- transduced	Negative	Negative	Negative	Negative
Cell mix homogeneity (VCN)	Cohort B:	RV: 0 LV: 0.00013	RV: 0 LV:0.000285	RV: 0 LV:0.000448	Not established
	Cohort C:	RV: 0.000395 LV: 1.73	RV: 0 LV: 2.9	RV: 0 LV: 1.95	
	Cohort D:	RV: 6.31 LV: 0.0051	RV: 5.87 LV: 0	RV: 7.27 LV: 0	
	Cohort E:	RV: 6.29 LV: 1.57	RV: 5.64 LV: 3.07	RV: 6.97 LV: 1.46	
Cell mix Viability before and after administration	Cohort B:	Before: 79.8% After: 77.3%	Before: 81.4% After: 73.4%	Before: 83% After: 82%	Not established
	Cohort C:	Before: 77.2% After: NA[f]	Before: 77.1% After: 73.9%	Before: 77.4% After: 74.8%	
	Cohort D:	Before: 78.8% After: 79.9%	Before: 81.2% After: 76.6%	Before: 82.4% After: 83%	
	Cohort E:	Before: 74.0% After: 75.1%	Before: 75.3% After: 72.5%	Before: 76.6% After: 79.4%	

Given the high number of animals included in the study, it was divided into 3 experiments. Animals in Experiments 1 and 2 were euthanized at 3 months after BMT, while those in Experiment 3 were euthanized at 5 days after BMT.

[a] Assessed by the frequency of CD3+ cells in the single cell population. Measured by flow cytometry.
[b] Assessed by the frequency of Lin- cells in the single cell population. Measured by flow cytometry.
[c] Assessed by the frequency of Lin-, ScaI + c-kit+ (LSK) cells in the single cell population. Measured by flow cytometry.
[d] As determined by no bacterial and fungal growth in a 14 day culture.
[e] Lin- purity assessment performed on the same day of the Lin- purification did not work. Lin- purity checking was repeated the following day, and the gating strategy was set based on the unstained samples. LSK staining is not reliable, due to extended culture. Deviation from the study protocol was filed.
[f] All cells were used for injection, so there was no sample available to assess the viability.
EU, endotoxin units; LV, lentivirus; RV, retrovirus; VCN, vector copy number.

Supplemental Table 7. List of protocol-specific organs.

Tissues for Evaluation	Weight (g)	Histopathology
Spleen (collect sterilely)	X, Whole spleen	X Half for Histopathology. Half for VCN and flow
Femur &Tibia (right limb) (collect sterilely)	NA	NA, used for VCN and flow
Sternum	NA	X, Decalcified
Thymus & associated Fat Pad	NA	X
Lungs	NA	X
Larynx, Thyroid Glands and Trachea	NA	X
Heart	X	X
Liver with Gall- Bladder	X	X + Gall bladder
Pancreas	NA	X
Stomach	NA	X
Duodenum	NA	X
Ileum	NA	X
Jejunum	NA	X
Cecum	NA	X
Colon	NA	X
Mesenteric Lymph Nodes	NA	X
Adrenal Glands	NA	X
Kidneys	X, Weigh together	X, Right cross section, Left longitudinal section
Urinary Bladder	X	X
Ovary-Females	X	X
Uterus	NA	X
Testis with Epididymis-Male	X	X
Seminal Vesicle, Prostate Gland, Coagulating Gland-Males	NA	X
Eye with Optic Nerve	NA	X
Brain (without olfactory bulbs)	X	X
Skin & Mammary Glands-Females	NA	X
Quadriceps Femoris (left)	NA	X
Femur &Tibia (left)	NA	X, Decalcified
Remainder of Carcass/Tissues	NA	NA
Gross Lessions (if observed)	NA	X

Supplemental Table 8. Manufacturing validation runs.

Test		Acceptance Criteria	HD1*	HD2*	HD3*	HD4	HD5
CliniMACS enrichment							
CD34+ cell recovery after CliniMACS® enrichment		≥40%	50%	50%	60%	74%	68%
CD34+ cell purity after CliniMACS® enrichment		≥50%	97.08%	87.94%	98.87%	98.10%	99.58%
In Process tests							
Sterility (Day 0)		Negative	Negative	Negative	Negative	Negative	Negative
Sterility (Day 1)		Negative	Negative	Negative	Negative	Negative	Negative
TNC viability		≥70%	98.8%	99.1%	99.0	94.7	99.5
Cell Product (CP)							
TNC recovery#		≥80%	131%	94%	125%	59%	106%
% CD34+ cell purity		≥50%	98.66%	93.14%	97.68%	94.14%	96.27%
Vector copy number[&]		0.1-2 copies/cell	0.1	0.1	0.3	0.4	0.4
Sterility	Bacterial culture	Negative	Negative	Negative	Negative	Negative	Negative
	Fungal culture	Negative	Negative	Negative	Negative	Negative	Negative
Endotoxin concentration		≤ 5 EU/Kg (HD weight)	0.095	0.132	0.139	0.129	0.101
Mycoplasma contamination		Ratio<1	0.18	0.4	0.46	0.23	0.07
CFU assay		Report results	51±14%	64±4%	52±10%	36±1%	46±10%
% LV-NYESO+ CFUs		Report results	35.4%	22.4%	28.7%	43%	37%
$V_{\beta}13.1$** expression		Report results	18.8%	13.2%	24.8%	30.9%	31.4%

*HD1, HD2 and HD3 were manufactured with GMP-comparable grade vector.

#TNC recovery was calculated by dividing the number of the total nucleated cells (TNC) cells seeded for stimulation (Day 0) by the number of TNC harvested on Day 2 and multiplying by 100%.

&Analyses performed after cells are cultured for 14 days to avoid over estimating vector copy number (VCN) and potency due to the presence of un-integrated forms of the lentiviral vector.

** Vβ13.1-NYESO TCR variant of the TCR β chain

CFU, colony-forming units

Supplemental Table 9. Comparison between fresh and cryopreserved product at 1, 30, 90 and 180[#] days.

Test	Method	Specifications	FCP* (n=4)	Days post-cryopreservation				P value
				TCP* 1d (n=2)**	TCP 30d (n=5)	TCP 90d (n=3)	TCP 180d (n=2)	
%CD34 cells (purity)	Flow Cytometry (ISHAGE)	≥50%	95.9±2.7	95.9±0.5	95.6±3.4	96.5±0.5	97.5±0.5	0.95
%TNC viability	Trypan Blue manual count	≥70%	96.0±4.9	93.6±3.4	95.4±5.4	97.1±1.5	97.7±0.4	0.17
%CD34+ cell recovery	Flow Cytometry (ISHAGE)	Record results	83.2±14.4	90.5±9.6	89.5±5.4	97.7±20.3	83.2±14.4	0.31
Endotoxin concentration	Endosafe®PTS	≤ 5 EU/Kg	0.12±0.02	0.14±0.06	0.12±0.04	0.10±0.04	0.10±0.06	0.88
Mycoplasma contamination	MycoAlert test	Ratio<0.9	0.32±0.13	0.14±0.10	0.33±0.12	0.37±0.23	0.28±0.08	0.53
Sterility	Bacterial culture	No growth	No growth	No growth	No growth	No growth	No growth	N/A
	Fungal culture	No growth	No growth	No growth	No growth	No growth	No growth	N/A
%V_β13.1 positive cells	Flow Cytometry	Record results	22.0±7.7	32.0±0.8	26.6±1.2	19.5±1.2	9.5±0.5	0.15
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	51±11	40±9	45±9	48±8	49±4	0.45
%LV-NYESO TCR/sr39TK-positive CFU	ddPCR	Record results	29.2±5.9	40.0±4.2	31.7±9.2	20.5±5.7	25.9±0.1	0.15
Vector copy number (VCN)	ddPCR	Record results	0.2±0.2	0.4±0.0	0.3±0.1	0.2±0.1	0.2±0.1	0.8

[#]The numerical results in the table are mean ± SD. Linear mixed modeling approach was adopted for statistical analysis to evaluate the long-term stability of the cryopreserved cell products.

*FCP, fresh cell product; TCP, thawed cell product.

** This time point was assessed only for cell products manufactured with GMP-grade vector

SUPPLEMENTAL MATERIALS AND METHODS

Primary cells, viral vectors and mice

Human peripheral blood mononuclear cells (PBMCs) were obtained from leukapheresis products from healthy donors. The leukapheresis products were collected under UCLA Institutional Review Board (IRB) approval # 10-001598. The human CD34+ cells were purchased from AllCells (Alameda, CA) or HemaCare (Van Nuys, CA). Murine splenocytes and bone marrow cells were collected fresh from HLA-A2/K^b transgenic mouse donors (1). For the *in vitro* immortalization assay, bone marrow cells from C57BL/6J mice were used. HLA-A2/K^b transgenic mice (1) and C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were bred and housed under defined-flora, specific pathogen-free conditions at the AAALAC-approved animal facility of the Division of Experimental Radiation Oncology, UCLA, and used under the UCLA Animal Research Committee protocol #2013-095 that was approved in advance by the Institutional Animal Care and Use Committee.

The third-generation lentiviral vector encoding the codon-optimized α and β chains of the NY-ESO-1 TCR (derived from the 1G4 TCR clone(2)) together with the suicide gene sr39TK (RRL-MSCV-optNYESO-optsr39TK-WPRE) was generated by transient transfection in normal human kidney-derived HEK293T cells using the packaging plasmids (pMDL, pMDG1, and pRSV-Rev) and the vector plasmid (pRRL-MSCV-optNYESO-optsr39TK-WPRE). This vector was manufactured at the Indiana University Vector Production Facility (IUVPF, Indianapolis, IN) at GMP-comparable (cGMP) and GMP-compliant grades. Briefly, supernatant from the transfected cells was harvested, filtered, benzonase-treated to remove residual plasmid DNA, purified by Mustang Q ion exchange, concentrated by tangential flow filtration and formulated in X-VIVO 15 serum-free medium. For the GMP-grade vector, all assays required by the FDA to ensure the absence of adventitious agents and replication-competent lentiviruses were performed

(Supplemental Tables 1-2). The control lentiviral vectors encoding the codon-optimized α and β chains of the NY-ESO-1 TCR alone (RRL-MSCV-optNYESO-WPRE) and the empty lentivirus control (RRL-MSCV-empty-WPRE) were provided by D.B.K. and R.P.H. These vectors were manufactured at research grade by transient transfection in HEK293T cells using the packaging plasmids pCMV-dR8.91 and pMDG-VSVG plasmids and the vector plasmids (pRRL-MSCV-optNYESO-WPRE and pRRL-MSCV-empty-WPRE) as previously described (3). The ecotropic retroviral vector MSGV1-A2ab-1G4A-Ly3H10 was manufactured at IUVPF at cGMP grade by transient transfection in HEK293T cells of the vector plasmid pMSGV1-A2ab-1G4A-Ly3H10 (a kind gift from Dr. Paul F. Robbins and Dr. Steven A. Rosenberg) and the pCL-Eco plasmid (kind gift from Dr. Inder Verma (Addgene plasmid # 12371)(4) encoding for retroviral Gag, Pol and the ecotropic envelope proteins. The ecotropic SF91-eGFP-RRE retroviral vector was manufactured at IUVPF using a polyclonal GP+E producer cell line generated by D.B.K and R.P.H.

Peripheral blood mononuclear cell (PBMC) activation, transduction and expansion

Healthy donor PBMCs were thawed and activated for two days in RPMI (Corning, Corning, NY) media with 10% fetal bovine serum (FBS, Omega Scientific, Tarzana, CA), 50ng/ml OKT-3 (Miltenyi Biotec, Bergisch Gladbach, Germany) and 300 IU/mL rhIL-2 (Proleukin; Prometheus Laboratories, San Diego, CA). Two days after activation, PBMCs were either mock-transduced or transduced with the LV-NY-ESO-1 TCR/sr39TK at a multiplicity of infection (MOI) of 100 ifu/cell in retronectin-coated plates (Takara Bio USA, Inc, Mountain View, CA). PBMCs were expanded for 3 days. After expansion, the expression of the NY-ESO-1 TCR at the cell surface was measured by NY-ESO-1(157-162)_(SLLMWITQV)-PE and matching HLA-negative dextramer (Immudex, Copenhagen, Denmark) in conjunction with a CD3-Brilliant Violet (BV)-650, CD4-BV510, CD8-BV605 (Biolegend, San Diego, CA), as well as 7-Aminoactinomycin D (7AAD)

(Beckman Coulter, Brea, CA) staining. Acquisition was done using two LSR II Flow Cytometers, both with four lasers (blue, red, violet, and ultraviolet; BD Biosciences, San Jose, CA). All flow cytometry data analyses were done with FlowJo v9 or v10 (Tree Star Inc., Asland, OR). Biexponential displays were used in the analyses.

CD34+ cell enrichment, stimulation, transduction, final formulation and cryopreservation.

The CD34+ cell population was enriched using the CliniMACS®CD34 reagent system (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer's instructions. The enriched CD34+ cell population was pre-stimulated overnight at a concentration of $1x10^6$ cells/mL in X-VIVO15 medium (Lonza, Walkersville, MD) supplemented with the following cocktail of GMP-grade recombinant human (rh) cytokines: rhSCF (50ng/mL), Flt-3 ligand (50ng/mL), TPO (50 ng/mL) and IL-3 (20ng/mL) (all from Miltenyi Biotec). After 18±6 hours of pre-stimulation, transduction of the peripheral CD34+ cells was performed in retronectin-coated plates using GMP-comparable or clinical-grade LV-NYESO TCR/sr39TK vector supernatant at a final MOI of 50 (two transduction cycles of 25 MOI each). After 18±6 hours from the first transduction, cells were harvested and formulated in cryopreservation solution consisting of Plasmalyte-A (Baxter Healthcare, Deerfield, IL); 5% DMSO (Cryoserv, Mylan Inc., Canonsburg, PA); 6% Pentastarch (Preservation Solutions Inc., Elkhorn, WI) and 5% human serum albumin (HSA; Grifols, Los Angeles, CA). The final cell product was cryopreserved using a controlled-rate freezer.

Suicide gene sr39TK activity

Human CD34+ cells mock-transduced and transduced with the lentivirus LV-NY-ESO-1 TCR/sr39TK were cultured for 72 hours. After expansion, the cells were harvested and seeded in

fresh basal media at $1x10^6$ cells/mL in 24 well-plates coated with retronectin (20µg/mL). Cells were then treated with ganciclovir (Gemini Bio-Products, Sacramento, CA) at concentrations of 0, 0.02, 0.2, 2, 20 or 200 µM for 48 hours. The percentage of cells co-expressing NY-ESO-1 TCR and sr39TK was assessed by flow cytometry analysis. Briefly, Zombie Violet Fixable Viability Kit (Biolegend, San Diego, CA) for exclusion of dead cells was used, following the manufacturer's instructions. Then, the cells were permeabilized using BD Cytofix/Cytoperm™ kit (BD Bioscience, San Jose, CA) and intracellular staining of the NY-ESO-1 TCR beta chain (anti-human TCR V_β13-PE, clone IMMU222 Beckman Coulter) was performed. FBS (Omega Scientific) was used to block. V_β13 isotype controls (IgG2b, κ isotype-PE, clone 27-35, BD Bioscience) were used to set the gates. Acquisition was performed as described above.

Lin- cell purification, transduction and expansion

The Lin- population was isolated from the total bone marrow using the Mouse Hematopoietic Progenitor Cell Enrichment (StemCell Technologies, Vancouver, Canada) negative selection kit. Briefly, HLA-A2/K^b transgenic mice (for the bone marrow transplantation (BMT) studies) or C57BL6/J mice (for the *in vitro* immortalization experiment) were euthanized, the femurs and tibias were collected and cleaned, and the bone marrow was recovered by flushing the marrow cavities with Robosep buffer (StemCell Technologies). Cells from multiple donors were pooled and resuspended at a concentration of $1x10^8$ cells/mL in Robosep buffer. The purification was performed in batches if more than $5x10^8$ cells needed to be processed. 50µL/mL of normal rat serum and 50µL/mL of EasySep Mouse Hematopoietic Progenitor Cell Isolation Cocktail (StemCell Technologies) were added to the cells and incubated for 30 min on ice. The cells were washed, and 100 µL/mL of EasySep Biotin Selection Cocktail (StemCell Technologies) was added for a 30-min incubation on ice. After incubation, 75 µL/mL of magnetic particles (StemCell

Technologies) were added and incubated for 10 min on ice. The EasySep Magnet (StemCell Technologies) was used to retain all of the lineage-positive cells. The fraction of cells not retained by the magnet was considered to be the Lin- cell fraction. To assess the purity, total bone marrow cells and the Lin- cell fraction were stained with antibodies for the lineage markers CD3e-FITC (Clone 145-2C11, eBioscience, San Diego, CA), CD45R-FITC (Clone RA3-6B2, eBioscience), CD19-FITC (Clone 6D5, StemCell Technologies), CD11b-FITC (Clone M1/70, eBioscience), Gr1-FITC (Clone RB6-8C5, eBioscience) and TER119-FITC (Clone TER-119, eBioscience) and the progenitor stem cell markers ScaI-PE-Cy7 (Clone D7, eBioscience) and cKIT-APC (Clone: 2B8, eBioscience). Purified CD16/32 (clone 93, eBioscience) was used to block. The percentage of Lin- cells and the frequency of ScaI+ and cKIT+ cells in the Lin- population were measured by flow cytometry.

After purification, cells were seeded at $0.5x10^6$ cells/mL in StemSpan SFEM media (Stem Cell Technologies) supplemented with L-glutamine, penicillin and streptomycin (Gemini BioProducts, West Sacramento, CA), 50ng/mL of mSCF (R&D Systems, Minneapolis, MN), 100ng/mL hIL-11 (R&D Systems), 20ng/mL mIL-3 (R&D Systems) and 100ng/mL of hFlt3-L (R&D Systems). The Lin- cells were pre-stimulated between 18-20 hours and 2 days. After pre-stimulation, the cells were transduced with the indicated lentiviral vectors using an MOI of 2-7.5 (an MOI of 7.5 ifu/cell was used for the GLP co-administration studies) and seeded in retronectin-coated plates. The cells were transduced and cultured for an additional 24-48 hours. Shorter pre-stimulation times (18-20 hours) and expansion times (24 hours) improved stem cell engraftment and long-term survival of the mice receiving BMT. Mock-transduced Lin- cells were manufactured in parallel to be used for the control groups.

T cell purification, transduction and expansion

The T cells were purified from spleens of HLA-A2/K^b transgenic mice. Briefly, the mice were euthanized, the spleens were collected in sterile conditions and a single cell splenocyte suspension was obtained by dissociating the spleens with a syringe plunger through a 70 µm porous mesh nylon strainer and then washing them with Robosep buffer (StemCell Technologies). The T cells were purified from the single cell suspension by negative selection using the EasySep™ Mouse T cell Isolation Kit (Stem Cell Technologies) according to the manufacturer's instructions. To assess the purity, splenocytes and purified T cells were stained with an anti-CD3e-FITC (Clone: 145-2C11; eBioscience) antibody. Purified CD16/32 antibody (clone 93; eBioscience) was used to block, and 7-amino-actinomycin-D (7-AAD, Beckman Coulter) was used as a viability marker. The analysis was performed by flow cytometry as descried above. After purification, the T cells were activated with CD3/28 beads (Invitrogen, Carlsbad, CA) using a 1:1 ratio and expanded for one day in RPMI media supplemented with 10% FBS, 0.05mM 2-mercaptoethanol (Invitrogen), penicillin-streptomycin (Invitrogen), 20mM HEPES (Corning), 1mM sodium pyruvate (Invitrogen) and 40 IU/mL of mIL-2 (Peprotech, Rocky Hill, NJ). The next day, T cells were collected and transduced with the RV-NY-ESO-1 TCR by spinoculation in retronectin-coated plates using a 1:1 mix of vector supernatant and fresh media. After transduction, cells were expanded for 3 more days. Mock-transduced T cells were manufactured in parallel to be used for the control groups.

Bone marrow transplantation in myelodepleted HLA-A2/K^b host mice

After Lin- and T cell purification, transduction and expansion, cells were collected, washed in PBS and resuspended in PBS at the final concentration needed for administration to the mice. The CD3/CD28 beads were removed from the T cell preps. As part of the acceptance criteria in the GLP studies, several parameters were measured: i) viability in each cell suspension was measured using AO/PI (acridine orange (AO) and propidium iodide (PI); Nexcelom, Lawrence, MA) and an automatic cell counter (Nexcelom Cellometer Auto 2000, Nexcelom); ii) samples of the cell supernatant were used for mycoplasma contamination testing using MycoAlert Mycoplasma Detection Kit (Lonza); iii) cell supernatant samples were sent to Labs, Inc. (Centennial, CO) for sterility testing; iv) a fraction of each cell type was kept in culture for two weeks for VCN determination; and v) a fraction of each cell preparation was cryopreserved in liquid nitrogen and archived as part of the reserved samples for the GLP studies.

For the co-administration GLP studies, each of the cell suspensions was prepared at twice the desired final concentration of cells after which Lin- and T cell preparations were mixed 1:1 in volume to generate the final cell preparations ready for administration to the mice. The final volume administered was 200µL per mouse, containing two million T cells and between 0.2 and 2 million Lin- cells. As part of the acceptance criteria for the GLP studies, several parameters were also measured in the cell mixtures: i) cell pellets were collected to measure the endotoxin concentration in the final product using the Endosafe-PTS system (Charles River Laboratories, Wilmington, MA); ii) cell pellets were used to measure the lentivirus and retrovirus VCN; and iii) the cell viability in the cell mixtures was measured with AOPI and an automatic cell counter before and after cell administration to the mice.

The single cell preparations or the mixtures of Lin- and T cells were administered intravenously in the lateral tail vein to HLA-A2/K^b mice that had previously received lethal total body irradiation

of 900cGy (J.L. Shepherd Mark I Irradiator, San Fernando, CA). To help improve mouse recovery after irradiation, water supplemented with 0.25 mg/mL amoxicillin (Biomox, Virbac AH, Inc., Fort Worth, TX) and DietGel 76A (ClearH2O, Westbrook, ME) was provided *ad libitum*. Clinical observations and body weight measurements were performed and documented at least twice a week for the duration of each experiment.

Necropsies and sample processing

After bone marrow transplantation, mice were followed for three months unless otherwise indicated. Five days or three months after BMT, mice were euthanized by CO_2 asphyxiation. Cardiac blood was collected and used for hematology, serum chemistry (only for the mice euthanized at three months after BMT) and VCN determination as determined by the study protocols. Blood samples for hematology and serum chemistry were processed by Quality Vet Lab (Davis, CA) for the GLP-compliant studies and the UCLA Division of Laboratory Animal Medicine (Los Angeles, CA) or IDEXX BioResearch, (West Sacramento, CA) for the non-GLP research studies. Blood smears were performed as a backup. The spleen and bone marrow were collected and single bone marrow cells and splenocyte single cell suspensions were prepared for VCN determination and cell phenotype characterization (only for the mice euthanized at three months after BMT). If full pathology analysis was needed, the protocol-specified organs indicated in Supplemental Table 7 were collected and weighed (for selected organs). Tissues for histopathologic assessment were sent in GTF™ Formalin Substitute (Stat Lab McKinney, TX) to HistoTox Labs (Boulder, CO) to be processed in a GLP-compliant manner. Sections stained with H&E were analyzed by an ACVP board-certified veterinary pathologist (B.B).

Vector Copy Number

At least $0.5x10^6$ Lin- and T cells (each cultured for 2 weeks), $1\text{-}3x10^6$ bone marrow cells and splenocytes and 100 µL of blood samples were used to extract DNA using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD). Vector copy number (VCN) was determined for all DNAs via digital droplet PCR (ddPCR; BioRad, Hercules, CA) using the primer/probe sets (Integrated DNA Technology, Coralville, IA) specific for the lentiviral (assessed in 2-week cultured Lin- cells, blood, bone marrow cells and splenocytes) and retroviral vectors (assessed in 2-week cultured T cells, blood, bone marrow cells and splenocytes). A primer/probe set for the mouse specific uc378 region was used as internal calibrator. The primers and probes are the following: for the retroviral vector, Retro NYESO1 F (CCGGGTTTAATCTGCTCATG), Retro NYESO1 R (AGGCTGCACAGCACAGGAGG), Retro NYESO probe (/56-FAM/TGAAGTTGG /ZEN/TGGCTCCGGATCC/3IABkFQ/); for the lentiviral vector, HIVU5 F (AAGTAGTGTGTGCCCGTCTG), HIVpsi R (CCTCTGGTTTCCCTTTCGCT), HIV1U5 probe (/6-FAM/CCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAG/Iowa); for the internal calibrator, uc378 F (CGCCCCCTCCTCACCATTAT), uc378 R (CATCACAACCATCGCTGCCT), uc378 HEX probe (5' HEX-TTACCTTGCTTGTCGGACCAAGGCA-3' Iowa Black). For human bulk CD34+ cell cultures and single CFUs, the cellular autosomal gene syndecan 4 (SDC4) was used as the internal calibrator using the following probes: SDC4 F (CAGGGTCTGGGAGCCAAGT), SDC4 R (GCACAGTGCTGGACATTGACA), SDC4 HEX (5' HEX-CCCACCGAACCCAAGAAACTAGAGGAGAAT- 3' Iowa Black). For the IVIM studies, a primer probe set amplifying the GFP transgene was used to measure the VCN of the cells transduced with SF91-eGFP-WPRE. The sequences or the primer probe set are eGFP616F (CTGCTGCCCGACAACCA), eGFP705R (GAACTCCAGCAGGACCATGTG) and eGFP653 FAM Probe (6FAM—CCCTGAGCAAAGACCCCAACGAGA—Iowa Black FQ).

Flow cytometry

The expression of the NY-ESO-1 TCR and the bone marrow and splenocyte phenotype characterization were assessed by flow cytometry three months after BMT. Briefly, surface staining for multiple T cell markers [anti-mouse CD3e-FITC (clone 145-2C11), anti-mouse CD4-APC (clone RM4-5; BD Bioscience), anti-mouse CD8a-APC-Cy7 (clone 53-6.7; BD Bioscience), anti-mouse NK1.1-BV421 (clone PK136; Biolegend)], B cells and granulocyte markers [anti-mouse IgM- PECy7 (clone 11/41; eBioscience), anti-mouse Ly-6G-APC (clone 12B-8C5; eBioscience), anti-mouse CD11b-APC-eFluor780 (clone M1/70; eBioscience), anti-human/mouse B220-FITC (clone RA3-6B2; eBioscience) and anti-mouse F4/80-BV421 (clone BM8; eBioscience)] and the NY-ESO-1 TCR beta chain (V_β13, anti-human TCR V_β13-PE, clone IMMU222; Beckman Coulter) was performed in splenocyte samples followed by cell permeabilization using BD Cytofix/Cytoperm™ kit (BD Bioscience) according to the manufacturer's instructions and intracellular staining of the NY-ESO-1 TCR beta chain (V_β13). Surface staining for lineage and stem cell progenitor markers [anti-mouse CD3e-FITC (clone 145-2C1; eBioscience), anti-mouse CD8a-FITC (clone 53-6.7; eBioscience), anti-mouse NK1.1-FITC (clone PK136; eBioscience), anti-mouse TCRb-FITC (clone H57-597; eBioscience), Anti-mouse γ/δTCR-FITC (clone UC7-13D5; eBioscience), anti-human/mouse B220-FITC (clone RA3-6B2; eBioscience), anti-mouse Ly-6G (Gr-1)-FITC (clone RB6-8C5; eBioscience), anti-mouse TER-119-FITC (clone TER-119; eBioscience), anti-mouse CD11b-FITC (Clone M1/70; eBioscience), anti-mouse IgM-FITC (Southern Biotech, Birmingham, AL), anti-mouse CD117 (c-Kit)-APC (clone 2B8; eBiosicence), anti-mouse Ly-6A/E (Sca-1)-PECy7 (clone D7; eBioscience), anti-mouse CD150-BV421 (clone TC15-12F12.2, Biolegend) and anti-mouse CD48-APC-eFluor780 (clone HM 48-1, eBioscience) and the NY-ESO-1 TCR beta chain (V_β13) was performed in bone marrow samples followed by cell permeabilization using BD Cytofix/Cytoperm™ kit and intracellular staining of the NY-ESO-1 TCR beta chain (V_β13). Purified anti-mouse CD16/32 (clone 93;

eBioscience) was used to block. $V_\beta 13$ isotype controls (IgG2b, κ isotype-PE, clone 27-35; BD Bioscience) and fluorescence minus one (FMOs) controls were used.

Surface and total expression of the NY-ESO-1 TCR was measured in murine T cells transduced with the retroviral vector. Surface staining was performed using the $V_\beta 13$ antibody according to manufacturer's instructions. For the total NY-ESO-1 TCR expression, after surface staining with the $V_\beta 13$ antibody, cells were permeabilized using BD Cytofix/Cytoperm™ kit and stained for intracellular $V_\beta 13$ using the same antibody. Purified anti-mouse CD16/32 (clone 93; eBioscience) was used to block and isotype IgG2b was used as a control. Acquisition and analysis was performed as described above.

In vitro immortalization assay

Lin- cells from C57BL/6J mice were transduced with LV-NY-ESO-1 TCR/ sr39TK or the SF91-eGFP-WPRE retrovirus control in retronectin-coated plates using MOIs of 25, 50, 335, 670, 1005 and 1675 for the lentivirus and 1, 5, 10 and 20 for the retrovirus. After transduction, cells were cultured for 2 weeks. The VCN was determined by droplet digital PCR (ddPCR) eight days after transduction. After mass expansion for 2 weeks, cells were plated in limiting dilution in 96 well plates at 100 cells/well and 1000 cells/well and cultured for an additional 2 weeks. The number of wells containing immortalized clones was counted and the replating frequency was calculated using ELDA extreme limiting dilution software (5) (http://bioinf.wehi.edu.au/software/elda) for each sample. The replating frequencies were normalized by the VCN.

CD34+ cell quality assessment tests

At least 10% of the CD34+ final cell product was reserved to test for sterility, endotoxin levels, mycoplasma contamination, clonogenic potential and transduction efficiency. Sterility was assessed by bacterial and fungal cultures for 14 and 21 days, respectively, at the Clinical Microbiology Labs (UCLA). Endotoxin levels were assessed by using the Endosafe®-PTS™ test system. Mycoplasma was assessed by the MycoAlert™ Mycoplasma Detection Kit. The number, purity and viability of CD34+ cells before/after enrichment and in the cell product were evaluated using the single-platform ISHAGE flow cytometric method (6). Intracellular $V_{\beta}13$ chain expression (TCR β chain variant specific for NY-ESO-1 TCR) was assessed by flow cytometry.

Colony-forming unit (CFU) potential was evaluated by culturing CD34+ cells in a methylcellulose-based medium MethoCult™ H4435 Enriched (StemCell Technologies). $1x10^2$ and $1x10^3$ viable cells were plated in duplicate in 35mm culture dishes and incubated for 13-15 days in a humidified 5% CO_2 incubator. Colonies were counted using an inverted phase contrast microscope and characterized based on their unique morphology (7).

The VCN of integrated lentivirus per cell of the bulk cell culture was assessed by plating 5×10^4 cells in Iscove's Modified Dulbecco's Medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA) supplemented with 20% (vol/vol) FBS (Omega Scientific), 1% (vol/vol) L-glutamine, and penicillin-streptomycin mixture (Gibco; Thermo Fisher Scientific, Inc) as well as rhIL-3 (5ng/ml), rhIL-6 (10 ng/ml) and rhSCF (25 ng/ml) (Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were cultured for 14 days. The average VCN per cell was determined by ddPCR, as described above.

Single CFUs were individually picked to identify the percentage of LV-NYESO TCR/sr39TK-positive CFUs. The genomic DNA was isolated using NucleoSpin® Tissue XS kit (Macherey-Nagel, Bethlehem, PA). The number of positive colonies was assessed by ddPCR.

LV-NYESO TCR/sr39TK PBSC stability evaluation

The long-term stability of the frozen cell product was evaluated after storage in the vapor phase of liquid nitrogen (≤-140°C) for 30±7, 90±7 and 180±10 days. To evaluate the post-thaw stability over a 48-hour period (short-term), the thawed cell product was split into two cryo-bags. One bag was stored at ambient room temperature and the other was refrigerated at 4-8°C in a temperature-monitored refrigerator. At each of the 3, 6, 24 and 48-hour time points, 1-1.5 mL samples were taken from each of the bags and evaluated for TNC numbers, viability, CD34+ cell numbers and CFU potential.

Differentiation of NYESO TCR/sr39TK-engineered T cells in ATOs (artificial thymic organoids)

Performed as described in (8).

Statistical analysis

Overall survival estimates of animals receiving BMT were obtained by Kaplan-Meier product-limit method and plotted by treatment cohorts. Comparison of overall survival between treatment cohorts was performed via the log-rank test. For quantitative outcomes including lentivirus/ retrovirus VCNs, splenocyte and bone marrow cell counts, frequencies of bone marrow cell subsets, frequencies of splenocyte cell subsets, body and organ weights, hematological and serum chemistry parameters, descriptive statistics (such as number of animals, mean, standard deviation, minimum and maximum by treatment groups) were calculated. Pair-wise comparisons of least-squares means in a linear model framework with Tukey-Kramer adjustment for multiple testing was used to measure the statistical significance. For the VCN analysis in the immunogenicity study (Figure 5), normality assumption was not met for the data and consequently

the pair-wise comparison analysis was performed using the non-parametric Dwass, Steel, Critchlow-Fligner method to adjust for multiple comparisons.

To assess the differences in the replating frequency/VCN between the SF91-eGFP-WPRE positive control and the LV-NY-ESO-1 TCR/ sr39TK, two different statistical tests were applied, Fisher's exact test for comparing the dichotomized positive/negative replating frequency/VCN and Wilcoxon rank sum test for the quantitative replating frequency/VCN.

A linear mixed modeling approach was adopted to evaluate the long-term stability and short-term stability of the cryopreserved cell products. Specifically, donor-to-donor variations were modeled through random intercepts, while time was treated as a fixed effect. Within the linear mixed model framework, an overall test of time effect was performed, followed by Tukey-Kramer adjusted pairwise comparisons.

For all statistical investigations, tests for significance were two-tailed. A p-value less than the 0.05 significance level was considered to be statistically significant. All statistical analyses were carried out using SAS software (version 9.4; SAS Institute Inc. 2013).

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4. GLP study protocol

PROTOCOL

Study Title:	**Effects of Co-Administration of NY-ESO-1 TCR genetically modified T cells and Hematopoietic Stem Cells (HSCs) in HLA-A2.1/K^b mice**
Testing Facility:	Ribas Laboratory University of California, Los Angeles Division of Hematology/Oncology David Geffen School of Medicine 10833 Le Conte Ave., Factor Building 9-954 Los Angeles, CA 90095 Division of Laboratory Animal Medicine (DLAM) University of California, Los Angeles Division of Laboratory Animal Medicine David Geffen School of Medicine 10833 Le Conte Avenue, Room 16-222H CHS Box 957372, Los Angeles, CA 90095
Study Director:	Cristina Puig Saus, PhD Postdoctoral Fellow University of California, Los Angeles, CA
Sponsor:	Antoni Ribas, MD, PhD Professor of Medicine University of California, Los Angeles, CA
UCLA IACUC (ARC) #:	2013-095
UCLA IBC #:	58.12
UCLA Study #:	GLP 16-001-RL

SIGNATURES

Study Director Date
Cristina Puig Saus, PhD

Test Facility Management / Vice Dean for Research Date
Stephen Smale, PhD

Sponsor / Professor of Medicine and Surgery Date
Antoni Ribas, MD, PhD

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1.0 KEY PERSONNEL

Study Director	Cristina Puig Saus, PhD Postdoctoral Fellow Telephone No.: 310-500-5735 E-mail: cpuigsaus@mednet.ucla.edu
Test Facility Liaison (Ribas Laboratory)	Paula Kaplan-Lefko, PhD Project Manager Telephone No.: 310-206-2090 E-mail: PKaplanLefko@mednet.ucla.edu
Test Facility Liaison (DLAM)	Jeffrey Goodwin, DVM, PhD, DACLAM DLAM Executive Director Telephone No.: 310-794-0286 E-mail: JGoodwin@mednet.ucla.edu
Project Scientist	Giulia Parisi, PhD Postdoctoral Fellow Telephone No.: 424-744-9344 E-mail: gparisi@mednet.ucla.edu
Project Scientist	James McCabe Animal Health Technician Telephone No.: 805-341-2898 E-mail: jmccabe@mednet.ucla.edu
Project Scientist	Salemiz Sandoval, PhD Assistant Project Scientist Telephone No.: 626-780-8379 E-mail: salemizsandoval@mednet.ucla.edu
Test Facility Veterinarian	Joanne Zahorsky-Reeves, DVM, PhD, DACLAM Senior Clinical Veterinarian Telephone No.: 310-794-0255 E-mail: jreeves@mednet.ucla.edu
Test Facility Veterinarian (backup)	Cristobal Torres, DVM, MPH Clinical Veterinarian Telephone No.: 310 694 7210 E-mail: ctorresurbano@mednet.ucla.edu

Quality Assurance Unit	Denise Breazeal Quality Assurance Unit Telephone No.: 310-825-2845 E-mail: dbreazeal@mednet.ucla.edu
Biostatistician	Xiaoyan Wang, PhD Adjunct Assistant Professor Telephone No.: 310-794-3114 E-mail: xywang@mednet.ucla.edu

2.0 TEST SITES

Test Site for Sterility Test

Company	Labs Inc. 6933-B S Revere Pkwy Centennial, CO 80112
Primary Contact for Sterility Testing	Monroe A. Burgess, CTBS Regional Manager - West Telephone No.: 520.990.1573/ 800.321.6088 E-mail: Monroe_Burgess@labs-inc.org

Test Site for Hematology and Serum Chemistry

Company	Quality Veterinary Laboratory 2121 2nd Street, C-104 Davis, CA 95618
Principal Investigator for Hematology and Serum Chemisty	Joan Shewmaker CLS, MT(ASCP) Manager Telephone No.: 530-759-8533 Fax: 530-759-8553 E-mail: joan@qualityvetlab.com
Quality Assurance Contact	Marvin Chaw QA Consultant Telephone No.: 530-759-8533 E-mail: emcee3consulting@gmail.com

Test Sites for Histology and Anatomic Pathology

Company (histology)	HistoTox Labs, Inc. 2108 55th St, Suite 110 Boulder, CO 80301
Primary Contact for Histology Processing	Jon Bishop President HistoTox Labs, Inc. Telephone No.: 303-633-5401 E-mail: jbishop@histotoxlabs.com
Principal Investigator for Anatomic Pathology	Brad Bolon, DVM, MS, PhD, DACVP, DABT, FATS, FIATP GEMpath, Inc. 1100 East 17th Avenue, Unit M202 Longmont, CO 80504 Telephone No.: 720-209-1105 E-mail: bradgempath@aol.com
Quality Assurance Contact for Histology and Anatomic Pathology	Kendy L. Keatley, BS HistoTox Labs, Inc. Contract QA 2200 24th Ave. Longmont, CO 80501 Telephone No.: 303-485-3876 E-mail: klkeat@msn.com

3.0 PROPOSED STUDY TIMETABLE

Experimental Start Date	27th April 2016
Cell Therapy Manufacturing	Experiment 1: 9th May – 13th May 2016 Experiment 2: 16th May – 20th May 2016 Experiment 3: 20th June – 24th June 2016
In-life Start Date	Experiment 1: 13th May 2016 Experiment 2: 20th May 2016 Experiment 3: 24th June 2016
In-life End Date	Experiment 1: 8th -9th August 2016 Experiment 2: 15th -16th August 2016 Experiment 3: 29th June 2016

Experimental Termination Date	November 2016
Unaudited Draft Report Date	November 2016
Study Completion Date	Date the Study Director signs the final report

4.0 OBJECTIVES

The goal of this study is to assess the safety of the co-administration of T cells transduced with retrovirus encoding NY-ESO-1 T cell receptor (TCR) and hematopoietic stem cells (HSCs) transduced with lentivirus encoding NY-ESO-1 TCR/sr39TK in HLA-A2.1/K^b mice. Safety of co-administration will be compared to that observed in animals receiving control mock transduced HSCs and mock transduced T cells, mock transduced HSCs and transduced T cells, transduced HSCs and mock transduced T cells and animals receiving no treatment. The parameters to be assessed include: 1) the effect of co-administration on engraftment of transduced T cells and HSCs and 2) the effect of co-administration on hematopoietic cell lineage development.

5.0 JUSTIFICATION

We and others have conducted adoptive cell therapy clinical trials using T cells transduced with a retroviral NY-ESO-1 TCR vector. We plan to now test the use of NY-ESO-1 TCR transduced T cells in combination with hematopoietic stem cells (HSCs) transduced with a lentiviral NY-ESO-1 TCR/sr39TK vector. The purpose of this toxicity study is to assess:

- The safety of the co-administration of this combination since it is possible that the transduced T cells and transduced HSCs could interfere with the engraftment of the other cell type or that co-administration of T cells and HSCs together could adversely affect hematopoietic cell lineage development.

6.0 REGULATORY COMPLIANCE

The experiments described in this proposal will be performed at an academic biomedical research center (University of California, Los Angeles (UCLA)) in accordance with the United States Food and Drug Administration (FDA): Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies, Code of Federal Regulations, Title 21 Part 58. Relevant standard operating procedures (SOPs)/protocols will be followed.

Exceptions to GLPs include the following study elements.

- The sterility test to be performed by LABS, Inc., a comprehensive, highly accredited and licensed clinical reference testing laboratory, will be performed according to all requirements for clinical samples.

- The irradiator located in the Biological Sciences Research Building used to irradiate mice will not be calibrated. Standard operating procedures are used and biannual safety inspections are performed by EH&S (UCLA office of Environment, Health and Safety). A myelodepleting dose of radiation has been established for the mice used in this protocol in previous experiments, and myelodepetion will be assessed in the current protocol at day 5 after irradiation.

7.0 ANIMAL CARE AND USE STATEMENT

UCLA's animal research facilities are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures in this study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR: Parts 1, 2 and 3) and the *Guide for the Care and Use of Laboratory Animals* (National Research Council, National Academy Press, Washington, D.C., Copyright 2011).

8.0 VETERINARY CARE/PALLIATIVE AND PROPHYLACTIC MEASURES

In accordance with the Animal Welfare Act (U.S.C Title 7 Chapter 54 Transportation, Sale and Handling of certain animals and CFR Title 9 Chapter 1 Subchapter A Animal Welfare), the Guide for the Care and Use of Laboratory Animals (8^{th} edition, National Research Council) and the Office of Laboratory Animal Welfare, medical treatment necessary to prevent unacceptable pain and suffering, including euthanasia, is the sole responsibility of the clinical veterinarian. Supportive care such as saline injections will be provided to mice if signs of dehydratation or other toxicity associated with irradiation appear. Palliative and prophylactic procedures may be based upon consensus agreement between the study director and clinical veterinarian. The study director and sponsor/designee (if possible) will be included in discussions of palliative and prophylactic procedures recommended by the clinical veterinarian. The clinical veterinarian, the study director and the sponsor (if possible) will make all decisions concerning the health and wellbeing of the animals and any subsequent decision to euthanize them.

9.0 TEST AND CONTROL ARTICLES

The test articles for this study are HSC enriched lineage depleted bone marrow cells (Lin-) and splenic T cells obtained from HLA-A2.1/K^b mice that are transduced under one of the following conditions:

1) Lin- bone marrow cells transduced ex vivo with LV-NY-ESO-1 TCR/sr39TK lentiviral vector (test article 1)
2) T cells transduced ex vivo with RV-NY-ESO-1 TCR retroviral vector (EcoMSGV1-A2aB-1G4A-Ly3H10) (test article 2)
3) Lin- bone marrow cells and T cells cultured in their respective medium without lentiviral or retroviral vector (control articles).

Test articles and control articles will be combined according to Tables 1-2 to generate the different treatments.

Both vectors were produced at Good Manufacturing Practice (GMP)-Comparable grade at the Indiana University Vector Production Facility (IUVPF, Indianapolis, IN). The IUVPF will provide documentation on the lot number, sterility, endotoxin and mycoplasma assessment for inclusion in the final report. Identity of the vector will be assessed in the Sponsor's laboratory at UCLA, by sequencing the vector plasmid. Physical titer of the lentiviral vector will be assayed in the Sponsor's laboratory at UCLA. All information will be provided in a Certificate of Analysis (COA) or equivalent analysis report. Reports will be compiled and included in the final report.

Reserve Sample Collection for Archive

- Lin- cells transduced with LV-NY-ESO-1 TCR/sr39TK, at least 2 aliquots with at least 0.5 x 10^6 cells each stored in Liquid Nitrogen (test article 1).
- Lin- cells mock transduced, at least 2 aliquots with at least 0.5x 10^6 cells each stored in Liquid Nitrogen (control article).
- T cells transduced with RV-NY-ESO-1 TCR, at least 2 aliquots with at least 2 x 10^6 cells each stored in Liquid Nitrogen (test article 2).
- T cells mock transduced, at least 2 aliquots with at least 2 x 10^6 cells each stored in Liquid Nitrogen (control article)

Reserve samples will be archived no later than 30 days following the completion of the study.

10.0 **TEST SYSTEM**

Donor Species and Strain:	Species: Mouse, (*Mus* musculus) Strain: HLA-A2.1/K^b 125-235 female mice will serve as donors. The donors will be 4-12 weeks of age at the time of tissue harvest. 7-12 mice will be used as donors for T cell and 35-65 mice will be used as donors for Lin- cells in each experiment (see tables 1-2). Note: Additional animals will be bred in case additional donors are needed. Additional mice may be required based on the yield of cells from the initial tissue harvest.
Recipient Species and Strain:	Species: Mouse, (*Mus* musculus) Strain: HLA-A2.1/K^b Projected use of 110-200 mice (approximately 50% males and 50% females) will be assigned to the study. Additional mice may be added depending on external variables (eg.

health issues prior to the administration of the test and control articles or failed intravenous administration of the test and control articles). Experiments and cohorts are described in Tables 1-2 (see below). Recipient Mice will be 8-14 weeks of age at the time of transplant and at a minimum weight 18g for females and 20g for males.

Animal Source: HLA-A2.1/K^b mice (recipient and donors) obtained from the Radiation Oncology Department Breeding Colony maintained at UCLA. This colony has been tested for the HLA-A2.1/K^b transgene expression by HLA-A2.1 staining by flow cytometry. The colony is maintained in a homozygous condition. Test animals will be assessed for HLA-A2 expression at the 3 month time point.
Any mouse that is not in optimal health as defined by a pre-study physical examination will not be included in the study.

11.0 **ANIMAL HUSBANDRY**

Housing: Mice will be housed in individually ventilated, irradiated, disposable cages (Innovive Inc. San Diego, CA) supplied with HEPA filtered air. The cages will be filled with certified ALPHA-dri® Plus irradiated bedding (Newco, Rancho Cucamonga, CA). Males and females will be housed separately. The cage density will be 4 mice per cage unless animals need to be separated due to fighting or health concerns.

Acclimatization Period: 1-3 weeks. The acclimation period will start once the recipient mice are moved to the test facility (BSRB-B60C, Biological Sciences Research Building). The mice (all cohorts) will receive amoxicillin treated water (at a final concentration of 0.25 mg/ml) and certified irradiated rodent diet at this time. The age range of the hosts will be maintained independently of the acclimatization time.

Food: The mice will be fed certified, irradiated rodent diet (LabDiet inc., Cat# 5K75, LABDIET ADVANCED PROTOCOL CERTIFIED PICOLAB RODENT 20%, Newco, Rancho Cucamonga, CA) *ad libitum.* The certificates of analysis for the food will be archived as part of this study.

Water: The mice will be provided with non-acidified ultrapure water (tested and certified), delivered in pre-filled amber tint mouse water bottles (Aquavive®, Innovive Inc. San Diego, CA) *ad libitum*. The certificates of analysis for the water will be archived as part of this study. The water will be supplemented with amoxicillin (0.25 mg/ml) for the duration of the study. Amoxicillin is a broad-spectrum antibiotic that treats a wide array of bacterial infections and is commonly given prophylactically to irradiated mice. The Amoxicillin is pharmaceutical grade as required by the *Guide for the Care and Use of Laboratory Animals* (*Guide*), Eighth Edition (National Research Council 2011).

Contaminants No known contaminants are present in the diet, water, or bedding (if applicable) at levels that might interfere with this study. The feed, bedding and water will be certified by the vendor prior to shipment to the test facility and will meet the requirements in accordance with the United States Food and Drug Administration (FDA): Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies, Code of Federal Regulations, Title 21 Part 58. The certificates of analysis for these products will be archived as part of this study.

Personnel All personnel handling the animals will have received training in the specific GLP Study Protocol and in the GLP regulations. Husbandry staff will follow the procedures outlined in the approved DLAM SOPs.

Room maintenance Animal room maintenance will be in accordance with approved DLAM SOPs.

Environmental Conditions:

Room air, temperature and relative humidity:

Mice will be housed in individually ventilated racks. The rack air is HEPA filtered via the rack blowers. Room temperature will be maintained between 20-26°C (68-79°F) monitored and recorded daily. Room humidity will be maintained between 30-70% and will also be monitored and recorded daily. The test facility is an AAALAC accredited facility and meets all air quality standards stated in the *Guide for the Care and Use of Laboratory Animals* (*Guide*), Eighth Edition (National Research Council 2011). All facility maintenance and calibration records for DLAM will be maintained by DLAM and will not be archived as part of this study. Records of

the temperature, humidity and light cycle for the test facility (BSRB, B60C) will be filed as part of this study.

Light:

An automatically-controlled 12-hour light: 12-hour dark fluorescent light cycle will be maintained. Each dark period will begin at 18:00 hrs PST (+/- 15 minutes). Light will be monitored and records will be filed as part of the study.

Health Status:

All mice utilized for this study were produced onsite in the 6V-CHS, Radiation-Oncology Rodent Breeding Facility. This facility is a barrier facility with restricted access. All caging, feed and water are autoclaved and all personnel entering the facility are dressed in sterile personal protective equipment (PPE: jumpsuit, shoe covers, mask, head cover and sterile gloves). All animals are maintained in a ventilated rack with HEPA filtered supplied air and handled under laminar flow changing hoods. The mice are tested for all major murine pathogens biannually and the colony is maintained as a Specific Pathogen Free (SPF) colony. Health reports from the breeding colony for the previous 12 months from the date of study initiation will be filed as part of this study and will serve as an indicator of health status for the mice utilized on this study. The recipient mice will be moved from the breeding colony in sterile cages and transferred into irradiated, sterile, disposable cages upon arrival to the test facility in the BSRB building (room# B60C). All personnel entering the test facility will also be required to dress in sterile PPE (jumpsuit, shoe covers, mask, head cover and sterile gloves). No other animals will be housed in the test facility with the test animals. All mice will only be handled under a certified biological safety cabinet for all procedures including cage changing. No sentinel mice will be maintained in the animal holding room in accordance with DLAM policy in bio-containment areas. No sentinel mice will be maintained in the animal holding room in accordance with DLAM policy in bio-containment areas. Environmental monitoring will be monitored according to DLAM SOPs.

12.0 **EXPERIMENTAL DESIGN**

Cell Harvest and Transduction for Adoptive T-Cell Transfer

The spleens from the donor mice will be harvested and dissociated to obtain single cell splenocyte suspensions. T cells will be isolated using the mouse EasySep Mouse T cell Isolation Kit with manual separation (StemCell Technologies, Vancouver, Canada), or equivalent kit. Purity will be determined by flow cytometry (CD3 staining). The cells will only be used for this study if the purity of CD3 positive cells is higher than 75%. Viability and cell number will be assessed using an automatic cell counter or a manual counting method. T cells will be activated by co-culture with CD3/CD28 activating beads (e.g. Dynabeads Mouse T-Activator CD3/CD28 for T-Cell Expansion and Activation, Life Technologies, Carlsbad, CA) for 20-28 hours. Cells then will be transduced with an ecotropic retrovirus encoding NY-ESO-1 TCR by spinoculation on retronectin-coated plates, and expanded for 3 days prior to administration of the cells to the recipient mice. Sterility and mycoplasma presence will be tested in the cell media, endotoxin

level and vector copy number (VCN) will be determined directly in the cell mixtures (described in Tables 1-2) on the day of cell administration. Cells will be kept in culture for 2 weeks and transduction efficiency will be determined by ddPCR using primers specific for the retrovirus transgene. The 2 week culture is necessary to dilute out the unintegrated vector. Transduction efficiency will also be determined by surface and intracellular TCR staining at least 6 days after transduction.

Cell Harvest and Transduction for Stem Cell Transplant

Donor mice will be euthanized and bone marrow from femurs and tibias will be harvested. Enrichment of Lineage negative (Lin-) cells will be performed using The EasySep™ Mouse Hematopoietic Progenitor Cell Enrichment Kit with manual separation (StemCell Technologies, Vancouver, Canada) or equivalent kit. Purity and quality of the cells will be determined by flow cytometry (Lin-, Sca-1 and c-kit staining), cells will be used for this study if the percentage of Lin- cells is higher than 25%. Viability and cell number will be assessed using an automatic cell counter or a manual counting method. Lin- cells will be prestimulated with cytokines for 16-24 hours, transduced with lentivirus encoding NY-ESO-1 TCR/sr39TK and expanded for an additional 16-24 hours prior to administration to the recipient mice. Sterility and mycoplasma presence will be tested in the cell culture media. Endotoxin level and VCN will be measured directly using the T cell and Lin- cell mixtures (described in Tables 1-2) prepared the day of cell administration. Transduction efficiency will be determined after 2 weeks in culture by digital droplet PCR (ddPCR) using primers specific for the lentiviral psi packaging signal. The 2 week culture of cells is necessary to dilute out the unintegrated vector. The target vector copy number (measured after a 2 week culture) per cell is 0.6-3. Transduction efficiency will also be determined by intracellular TCR staining at least 4 days after transduction.

Irradiation of Recipient Mice

Before cell transplant, mice in cohort B, C, D and E, according to the experimental design (see Tables 1-2), will be irradiated with a lethal dose of 900 cGy in the J.L. Shepherd Mark I Irradiator in the Biomedical Science Research Building (BSRB) mouse facility. Mice will be transplanted with donor cells within 24 hours post irradiation. Additional mice will be irradiated to be used in the event of a failed intravenous injection of the cell treatment. These additional mice will have unique IDs and all procedures will be documented. These mice will be euthanized on the day of administration if they were not needed for the study.

Cell Transplantation

T cell and Lin- cell mixtures will be injected into the tail vein of recipient HLA-A2.1/K^b mice according to the groups outlined in Tables 1-2. Cells will be combined and administered together as a single injection to reduce variability in injection efficiency, and reduce pain and anxiety for the animals.

Experimental Design (Summarized in Tables 1-2)

Due to the large number of animals, the study will be divided into three experiments. Two experiments (1 and 2) will include all groups with 4 males and 4 females in each group. The end point for these experiments will be 3 months after the transplant. The last experiment (3) will include all groups with 3 males and 3 females per group and the end point will be at day 5 after transplant.

Table 1. Experiments 1 and 2

Group	XRT	Treatment		3 Month[#]
		T cells	HSCs	
Cohort A	No	No	No	4M/4F
Cohort B	Yes	2-3 x 10^6 Mock Transduced T cells	1-2 x 10^6 Mock Transduced Lin- cells	4M/4F
Cohort C	Yes	2-3 x 10^6 Mock Transduced T cells	1-2 x 10^6 TCR Transduced Lin- cells	4M/4F
Cohort D	Yes	2-3 x 10^6 TCR Transduced T cells	1-2 x 10^6 Mock Transduced Lin- cells	4M/4F
Cohort E	Yes	2-3 x 10^6 TCR Transduced T cells	1-2 x10^6 TCR Transduced Lin- cells	4M/4F

Table 2. Experiment 3

Group	XRT	Treatment		Day 5
		T cells	HSCs	
Cohort A	No	No	No	3M/3F

Cohort B	Yes	2-3 x 10^6 Mock Transduced T cells	1-2 x 10^6 Mock Transduced Lin- cells	3M/3F
Cohort C	Yes	2-3 x 10^6 Mock Transduced T cells	1-2 x 10^6 TCR Transduced Lin- cells	3M/3F
Cohort D	Yes	2-3 x 10^6 TCR Transduced T cells	1-2 x 10^6 Mock Transduced Lin- cells	3M/3F
Cohort E	Yes	2-3 x 10^6 TCR Transduced T cells	1-2 x10^6 TCR Transduced Lin- cells	3M/3F

[#]3 months = 90 days ± 7 days to accommodate scheduling with external test sites and allow enough time to process and ship all samples. Euthanasia at this time point will be split in two consecutive days due to the large number of animals used.
Mice from cohort A will not be irradiated. XRT = Irradiation.

If for any reason, on the day of transplant, there are not enough cells for all mice, the number of mice per group will be reduced, accordingly. The reduction in the number of mice will be compensated in the following experiment, if possible. This will be documented in the study records.

The numbers of mice proposed in Tables 1-2 are based on our primary comparison at 3 months post-transplant, in which we will evaluate the key parameters of hematology between Cohort C and Cohort E. According to our preliminary data, white blood cell (WBC) count in Cohort C had a mean of 6.5K/uL with a standard deviation of 1.7 K/uL. An equivalence test of means using two one-sided tests with sample sizes of N=16 (4M/4F each in Experiment 1 and Experiment 2) animals in each of Cohort C and Cohort E achieves 89% power at 5% significance level when the true difference in mean WBC between the two groups is 0.0 K/uL, the standard deviation is 1.7 K/uL, and the equivalence limits are -2.0 K/uL and 2.0 K/uL (corresponding to +/- 30% of the Cohort C). We are confident that with the proposed numbers of animals we will have sufficient power to analyze the data. With the numbers proposed in Table 3, a sample size of 6 in each cohort produces a two-sided 95% confidence interval with a distance from the mean of VCN to the limits that is on the order of 1.0XSD (standard deviation).

Mice from each cohort will be euthanized at 5 days (experiment 3) and at 3 months (experiment 1 and 2) post-transplant. The T cell signal following T cell transplantation has been demonstrated to peak at 5 days in the mouse (Koya, et al. 2010). Based on our previous experience, the repopulation of the peripheral blood cells by progeny derived from transplanted HSCs should occur by 3 months in mice. Based on previous experiments, 1-2 x 10^6 –Lin- bone marrow cells are needed for successful engraftment and survival of lethally irradiated mice (unpublished data).

And at least 2-3 x 10^6 T cells are required for a successful adoptive cell transfer in mice based on previous experience.

This study will determine whether the co-administration of HSCs and T cells transduced with lentiviral and retroviral vectors expressing the same NY-ESO-1 TCR alters the differentiation of different hematological cell types. In addition, this study will determine whether the co-administration of both cell types has an adverse effect on engraftment of HSCs and on persistence of T cells and HSCs derived progeny.

Studies using HSC transplantation need to take into account potential toxicity from the procedures, therefore we will compare the experimental groups to mock transduced mice. In addition, there is the potential for the acceleration of T cell development by introduction of a pre-arranged TCR thereby increasing T cell proportions in transduced mice. This potential phenomenon will also be addressed in this study.

Endpoints

Peripheral blood, bone marrow and splenocytes will be harvested at 5 days and 3 months post transplant. The following endpoints will be assessed:

Day 5:

- Presence of transduced T cells in splenocytes and blood using ddPCR specific retroviral element primers
- Presence of transduced HSCs in bone marrow and blood using ddPCR specific for the lentivirus packaging signal (psi).
- Hematology to confirm myelodepletion
- Body weight
- Spleen weight
- Clinical observations

3 Months:

- Engraftment of transduced HSCs and persistence of their progeny in the bone marrow, spleen and blood using ddPCR specific for lentiviral elements.
- Persistence of the transduced T cells in the spleen, the bone marrow and the blood using ddPCR specific for the retroviral vector.
- Assessment of cell lineages in peripheral blood via Cell blood counts and differential counts (hematology)
- Assessment of engraftment of transduced HSCs by flow cytometry in bone marrow cells using antibodies to detect intracellular TCR and surface stem cell markers (see section 15.13).
- Assessment of transduced T cell and transduced HSC progeny by flow cytometry in the spleen using antibodies to detect intracellular TCR and T cell, B cell and granulocyte surface markers (see section 15.13).
- Confirmation of transgenic mouse phenotype by surface HLA-A2 staining in the splenocytes
- Body weight

- Clinical observations
- Gross pathology (with selected organ weights, see Table 4)
- Histopathology (of protocol-specified tissues, see Table 4)
- Serum chemistry

13.0 ANIMAL IDENTIFICATION, RANDOMIZATION AND ASSIGNMENT

Recipient mice will be acclimated for 1-3 weeks in room BSRB-B60C following the transfer from the 6V-CHS UCLA colony. After transfer, all mice will be identified with a unique identification (ID) number and a cage number. These numbers will be consecutive throughout the whole study. Mice will be ear punched for identification. Mice will be marked with a sharpie on the tail as a secondary method of identification after therapy administration. Prior to irradiation and transplantation of cells, the recipient mice will be randomized by gender and by cage into treatment groups. Cages will be randomized into treatment groups using the random numbers function in an excel file. Mice will be weighed and clinical observations will be documented before the treatment starts. Any mouse that is not in optimal health condition before the treatment starts will be excluded from the study, euthanized and substituted with a healthy mouse. Additional mice (not assigned to any group) will be irradiated. These mice will only be used in case of a failed cell injection and euthanized if not needed after cell administration. Any mice receiving a failed cell administration will be euthanized as well. All procedures will be appropriately documented.

14.0 TRANSPLANTATION

Ex vivo Transduction of Lineage Depleted Mouse Bone Marrow Cells and T cells

Isolated T cells will be cultured and transduced with retrovirus encoding NY-ESO-1 TCR (see 6.0 Experimental Design above). Lin- cells will be cultured and transduced with lentivirus encoding NY-ESO-1 TCR/sr39TK (see 6.0 Experimental Design above).

On the day of administration, cells will be collected and washed. Cell counts and viability will be recorded. Viability over 65% is required to continue with the cell administration. Cells will be resuspended at the correct concentration and mixed according to Tables 1-2. Viability of cell mixtures will be measured before and after treatment administration to assess stability. The culture medium of transduced and mock Lin- and T cells will be tested for sterility and mycoplasma (by biochemical test). Each cell mix (according to Tables 1-2) will be tested for endotoxin (limit per treatment 5EU/Kg). Sterility testing will be performed at Labs, Inc. (Centennial, CO) in accordance with their SOPs. Mycoplasma and endotoxin testing will be performed in the Ribas lab. Lentivirus and retrovirus VCN will be determined in the cell mixtures to confirm that equivalent amounts of each cell type have been mixed appropriately.
Transduction efficiency in Lin- cells and T cells will be tested for vector copy number (VCN) after culture for a period of 12-14 days to dilute out unintegrated vector.

Shipping: Cell media samples will be shipped from UCLA to Labs, Inc. for sterility testing.

Monroe A. Burgess, CTBS
LABS INC
6933-B S Revere Pkwy
Centennial, CO 80112
Telephone No.: 520.990.1573/ 800.321.6088
E-mail: Monroe_Burgess@labs-inc.org

Acceptance criteria:

Test Item	**Acceptability Criteria**
T cell purity	>75%
Lin- purity	>25%
Lin- VCN (2 weeks after culture)	0.6-3
T cell viability (day of administration)	> 65%
Lin- cell viability (day of administration)	> 65%
Mycoplasma	Negative
Endotoxin	<5EU/Kg
Sterility	Negative

T cell and Lin- purity and viability results will be available before treatment administration. If any of the parameters fail, the study director and the sponsor will determine if the experiment shall proceed. Animals will be transplanted prior to the availability of VCN, mycoplasma, endotoxin and sterility data. If any of the parameters fail, the study director and the sponsor will determine if the experiment should be terminated or will proceed. Since mouse cells do not freeze/thaw well and lyse readily when thawed and since it takes time to evaluate transduction efficiency and sterility, we plan to inject cells fresh in this study at the risk of having to euthanize animals if the results do not meet the release criteria.

15.0 OBSERVATIONS, MEASUREMENTS, AND TERMINATION

15.1 Clinical Observations and health checks

During the acclimation period and throughout the study, the animals will be observed daily by animal care staff for morbidity and mortality.

Daily general health checks are performed by DLAM personnel per approved DLAM SOPs.

Clinical observations will be made by the Ribas lab study personnel at least twice a week including the day of euthanasia and more frequently if deemed necessary according to the mouse health status. Observations will include changes in skin and fur, eyes, mucous membranes and other signs of respiratory, circulatory and neurological dysfunction. Animals having ruffled fur, hunched posture, severe dyspnea, self-mutilation and/or reluctance to move upon stimulation will be evaluated and considered for humane euthanasia. Additional actions will be made by study personnel if clinical signs warrant such as necropsy. All decisions concerning the health

and wellbeing of the animals and any subsequent decision to euthanize them will also be made by the Study Director in consult with the veterinarian.

Several complications associated with the myelodepleting irradiation and the bone marrow transplant are expected. Irradiation causes animal morbidity through tissue damage, which in turn elicits an inflammatory response. The symptoms of myeloablation in mice is weight loss (greater than 20% in some individuals), general lethargy, dehydration (ridged skin, spikey fur, sunken eyes). Mice that survive lethal total body irradiation and are able to reestablish their hematopoietic system after a bone marrow transplant will exhibit the symptoms of myeloablation but will recover during weeks 4-6 after irradiation. Recovery (a successful bone marrow transplant) is physically manifested by weight gain, return to normal activity levels, normal food and water consumption, normal defecation and urination volumes. The only residual effect that may be observed in a small percent of survivors (post irradiation) is a change in fur color from black to white or gray. This change in coloration may be isolated in patches or may involve the animal's entire body. In some cases, mice never recover, these mice will be euthanized.

15.2 Body Weight

Body weight will be recorded for all mice the day prior to irradiation, three times weekly until day 60 after bone marrow transplant, and then twice weekly until the time of scheduled euthanasia. Body weight will be recorded on additional days by study personnel if clinical signs warrant. Body weight on the day of euthanasia will be recorded prior to blood collection and necropsy.

15.3 Evaluation of Engraftment and Persistence of Gene Modified Cells Post Transplant

Mice in cohorts A-E will be euthanized at 5 days and 3 months post transplantation.

Day 5:

- Presence of transduced T cells: Splenocytes and peripheral blood will be harvested and VCN will be determined by ddPCR using primers specific for the retrovirus.
- Presence of transduced HSCs: Bone marrow and peripheral blood will be harvested and VCN will be determined by ddPCR using primers specific for the lentivirus Psi signal.

3 Months:

- Engraftment/persistence of transduced HSCs and their progeny: Bone marrow, splenocytes and blood will be harvested, and VCN will be determined by ddPCR using lentiviral element primers.
- Persistence of transduced T cells: Bone marrow, splenocytes and blood will be harvested, and VCN will be determined by ddPCR using retroviral element primers.

- Engraftment of transduced HSCs will be assessed by intracellular TCR and surface stem cell marker staining by flow cytometry of bone marrow cells (see section 15.13).

15.4 Evaluation of Hematopoietic Cell Lineage Development Post Transplant

Complete blood counts (CBC) with differential counts will be assessed in the peripheral blood from mice at 5 days and 3 months after transplant. Untreated mice (cohort A) will be also euthanized at 5 days and 3 months post transplantation, and peripheral blood will be collected for comparison to the other groups. CBC data at day 5 will be used to confirm myelodepletion. Hematology data 3 months after bone marrow transplant will be used to confirm the expected differentiation of the HSC into all blood cell subsets. CBC includes: WBC; relative and absolute values for neutrophils, lymphocytes, monocytes, eosinophils, and basophils; RBC, Hb, HCT, MCV, MCH, MCHC, RDW, PLT and MPV. Blood will be sent to Quality Vet Lab (Davis, CA) for CBC assessment to be performed under GLP regulations per their SOPs.

To assess the differentiation of transduced HSC into the different hematopoietic cell lineages, intracellular TCR together with T cell, B cell and granulocyte surface markers will be stained and analyzed by flow cytometry in splenocytes from mice euthanized 3 months after transplant (section 15.13).

15.5 Necropsy and Tissue Harvest

- Mice will be euthanized using CO_2 inhalation and subsequent exsanguination.
- A body weight will be recorded for each animal before euthanasia except as noted below (Unscheduled termination).
- Blood will be collected via cardiac puncture. Blood will be distributed and processed for hematology, chemistry and VCN as follows. Briefly, whole blood (minimum 350 µL at day 5 after transplant and 250µL at 3 months after transplant) will be collected in EDTA-containing tubes for evaluation of complete blood count (CBC) with differentials (see table 5). An aliquot of 100µL will be collected and transferred into an Eppendorf tube for VCN analysis. During the necropsy at 3 months after transplant, the remaining blood will be placed in a serum separator tube for serum chemistry evaluation. At least 400 µL of total blood is needed for this purpose. Chemistry analysis includes: ALT, AST, BUN, CRE-S, CK, ALP, BUN/CRE-S, GLU, ALB, TPRO and TBILI, GLOB and A/G. If enough blood is not obtained due to technical issues, samples will be distributed following the prioritization list stated in section 15.8. Also if there is not a sufficient amount of blood for hematology analysis, a white blood count estimate and a differential will be obtained from a blood smear (see section 15.10).
- Necropsies will consist of a complete external and internal examination including examination of body orifices (ears, nostrils, mouth, anus, etc.) and intra-cranial, thoracic and abdominal organs and tissues. The organs to be weighed and collected during necropsy are listed in Table 4. All gross findings will be recorded in descriptive terms including location, size, shape, color, consistency and number. Necropsies will be performed by trained prosectors.

15.6 Scheduled Termination

Day 5:

- A complete necropsy will NOT be performed.
- Blood will be collected for CBC.
- Blood, splenocytes and bone marrow will be harvested for VCN assessment.

3 Months:

- A complete necropsy will be performed on all animals (see section 15.5). Tissues will be processed and stained for histopathological examination by Histo Tox Labs, (Boulder, CO) as a GLP compliant study according to their SOPs and analyzed by anatomic pathologist Brad Bolon, DVM, MS, PhD, an ACVP board-certified veterinary pathologist. All tissues listed in Table 4 will be fixed by immersion in GTF™, Formalin Substitute solution for histopathology and send within a week to HistoTox Labs for further processing.
- Blood will be collected for serology (CBC and serum chemistry) and sent to Quality Vet Lab (Davis, CA).
- Blood, splenocytes and bone marrow will be harvested for VCN assessment.
- Splenocytes and bone marrow will be harvested for flow cytometry.

15.7 Unscheduled Termination and Death

Animals found dead:

When an animal is found dead in the cage, the carcass will be bagged, labelled with the animal's identification information, Sponsor Name, GLP Study Number, and stored in a refrigerator in B70B hallway until it can be necropsied by Ribas Lab trained personnel. The staff member who finds the dead animal will notify the Study Director immediately via phone and document notification of finding with an email to the Study Director.

- Since the exact time of death is unknown a modified necropsy will be performed. Blood samples will be omitted from the necropsy process of mice found dead in the cage.
- All organs listed in Table 4 will be collected if possible and fixed in GTF™, Formalin Substitute solution. These organs will be shipped for further processing to HistoTox Labs together with all the samples from scheduled necropsies.
- The carcasses will be kept in GTF™, Formalin Substitute solution and archived.
- Body and organ weights will not be assessed.

Animals euthanized prior to the scheduled necropsy:

Animals will be euthanized prior to the scheduled necropsy if clinical signs warrant it.

- For animals that need to be euthanized for humane reasons before the scheduled necropsy end point, a full necropsy will be performed whenever possible as described in section 15.5, and justification will be documented if necropsy is not possible. Samples for VCN analysis in blood, splenocytes and bone marrow cells will only be collected if trained personnel are available. Flow analysis will not be performed in these animals. If an animal is found moribund on a Friday, the weekend or a holiday blood samples will be refrigerated, a blood smear will be performed and kept at room temperature and serum samples will be kept at -80ºC. All these samples will be sent as soon as possible to Quality Vet Labs. If blood samples are not viable by the time it can be analyzed, a white count estimate and a differential will be assessed from the blood smear.

15.8 Tissue Sample Distribution

The distribution of tissue samples is summarized in Table 4. All major tissues from all necropsied mice will be harvested and analyzed as described in table 4 and following sections 15.9, 15.12 and 15.13.

Table 4. Distribution of Tissues at Necropsy for All Mice

Tissues for Evaluation	Weight[b]	Histopathology	ddPCR	Flow
Spleen Collect sterilely	X Whole spleen	X[c]	X[a,c]	X[a,c]
Right Femur &Tibia Collect sterilely	NA	NA	X[a]	X[a]
Sternum & Xiphoid process	NA	X, Decalcified		
Thymus & Associated Fat Pad	NA	X		
Lungs & Trachea	NA	X		
Larynx &Thyroid Gland	NA	X		
Heart	X	X		
Liver with Gall-Bladder attached	X	X + Gall bladder		
Pancreas	NA	X		
Stomach	NA	X		
Duodenum	NA	X		
Ileum	NA	X		
Jejunum	NA	X		
Cecum	NA	X		
Colon	NA	X		
Mesenteric Lymph	NA	X		

Nodes				
Adrenals	NA	X		
Kidneys	X Weigh together	X, Right cross section, Left longitudinal section		
Urinary Bladder	X	X		
Ovary-Female	X	X		
Uterus-Female		X		
Testes with epididymis-Male	X	X		
Seminal vesicles, Prostate Glands Coagulating Gland-Males	NA	X		
Eye with optic nerve	NA	X		
Brain (without olfactory bulbs)	X[d]	X		
Skin & Mammary Glands-(mammary glands for Females only)	NA	X		
Left Quadriceps Femoris	NA	X		
Left Femur &Tibia	NA	X, Decalcified		
Remainder of the Carcass/Tissues	NA	NA		
Gross lessions (if observed)	NA	X		

[a] Cell suspensions will be prepared and numbers and viability of cells will be assessed using an automated or manual cell counter. DNA will be isolated from approximately 1-5 x 10^6 cells for ddPCR analysis. Approximately 1-6 x 10^6 will be used for flow analysis.
[b] Weight will be recorded if the mice are terminated and not found dead.
[c] One half of the sample is designated for ddPCR and flow and the other half will be processed for histopathology.
[d] Brain will be sectioned according to Society of Toxicologic Pathology recommendations (Bolon et al 2013)
NA. Not assessed.

For tissues with limited materials, the testing will be prioritized as follows:

Peripheral Blood:

1. Hematology

2. Vector copy number (VCN)
3. Serum chemistry

Spleen:

1. ddPCR
2. Histopathology
3. Flow cytometry

Bone Marrow:

1. ddPCR
2. Histopathology
3. Flow cytometry

15.9 Histopathology

Tissues to be shipped to Histo Tox Labs, Inc. (Boulder, CO) and processed for histopathology according to GLP regulations per HistoTox SOPs on all mice in all groups are listed in Table 4. Tissues will be shipped in GTF™, Formalin Substitute solution. Remaining carcasses will be archived. Fixed tissues will be trimmed and embedded in paraffin and processed for histopathology. Slides will be analyzed by pathologist Dr. Brad Bolon either at the Histo Tox Labs facility (Boulder, CO) or at his office (Longmont, CO), as defined by availability of space at the Histo Tox Labs site when the slides are completed.

Shipping

Tissues will be shipped from UCLA to Histo Tox Labs, Inc for processing into slides.

Jon Bishop
HistoTox Labs, Inc.
2108 55th St, Suite 110
Boulder, CO 80301
Telephone No.: 303-633-5401
E-mail: jbishop@histotoxlabs.com

Slides and blocks will be maintained at Histo Tox Labs, Inc. The anatomic pathologist will review the slides either at HistoTox Labs, Inc or if necessary transport them to his nearby office.

Brad Bolon, DVM, PhD
GEMpath, Inc.
1100 East 17th Avenue, Unit M202
Longmont, CO 80504
Telephone No.: 720-209-1105
E-mail: bradgempath@aol.com

Following microscopic evaluation procedures, slides and blocks will be returned to the test facility prior to finalization of the report. Slides will be returned to the following address.

Cristina Puig Saus, PhD
David Geffen School of Medicine at UCLA
Department of Medicine
Division of Hematology/Oncology
10833 Le Conte Ave.
Factor Building 9-666
Los Angeles, CA 90095
E-mail: cpuigsaus@mednet.ucla.edu
Telephone No: 310-267-0596

The Principal Investigator for Anatomic Pathology will be responsible for all delegated phase activities and will submit a pathology sub-report from the data generated including hematology and serum chemistry interpretation, to the Study Director for inclusion in the final study report. The pathology report also will include a signed Good Laboratory Practices compliance statement and a Quality Assurance Statement.

15.10 Hematology and Serum Chemistry

Blood (as much as possible) will be collected via cardiac puncture prior to initiation of the full necropsy. Hematology and serum chemistry evaluations will be performed on blood samples collected from all designated mice scheduled for euthanasia and will be attempted on moribund animals (refer to section 15.7, Unscheduled Termination and Death for details). Blood will be collected, shipped and analyzed by Quality Vet Lab (Davis, CA) according to GLP regulations per Quality Vet Lab SOPs. For hematology analyses, whole blood will be placed into one tube per animal containing ethylene diamine tetraacedic acid (EDTA) as an anticoagulant. A blood smear will be prepared from each blood sample. Hematology parameters to be analyzed are shown in Table 5. If there is not enough sample to perform a hematology assessment, a differential blood count will be performed with the blood smear. For Serum chemistry blood will be placed in a serum separator tube and centrifuged to obtain the serum.
If enough blood can not be obtained for all analysis, then the priority listed in section 9.7 will be applied.

Day 5:

Only hematology samples will be collected at Day 5 termination.

3 Months:

Both hematology and serum chemistry will be collected at the 3-month termination.

Table 5. Hematology Parameters[a]

Parameter	Abbreviation	Units
Red Blood Cell Count	RBC	$10^6/\mu L$
White Blood Cell Count	WBC	$10^3/\mu L$
Hemoglobin	HGB	g/dL
Hematocrit	HCT	%
Mean Corpuscular Volume	MCV	fL
Mean Corpuscular Hemoglobin Concentration	MCHC	g/dL
Mean Corpuscular Hemoglobin	MCH	Pg
Red Cell Distribution Width	RDW	%
Platelet Count	PLT	$10^3/\mu L$
Mean Platelet Volume	MPV	fL
Relative Differential White Blood Cell Count		
Neutrophils	NEUT	% WBC
Lymphocytes	LYM	% WBC
Monocytes	MONO	% WBC
Eosinophils	ESO	% WBC
Basophils	BASO	% WBC
Absolute Differential While Blood Cell Count		
Neutrophils	Abs. NEUT	$10^3/\mu L$
Lymphocytes	Abs. LYM	$10^3/\mu L$
Monocytes	Abs. MONO	$10^3/\mu L$
Eosinophils	Abs. EOS	$10^3/\mu L$
Basophils	Abs. BASO	$10^3/\mu L$

[a] Abbreviations from the hematology system readout may differ from those listed above, but the results will be reported as described above.

For serum chemistry analyses, the remaining whole blood will be placed in a serum separator or clot tube for centrifugation to separate cellular and serum fractions. The serum chemistry parameters to be measured or calculated are shown in Table 6.

Table 6. Serum Chemistry Parameters[a]

Analyte	Abbreviation	Units
Alanine Amino Transferase (Alanine Transaminase) - Serum	ALT	IU/L
Aspartate Animo Transferase	AST	IU/L

(Aspartate Transaminase) - Serum		
Blood Urea Nitrogen	BUN	mg/dL
Creatinine (Serum)	CRE-S	mg/dL
Creatinine Kinase*	CK	IU/L
Alkaline Phosphatase*	ALP	IU/L
Blood Urea Nitrogen/Creatinine (Serum) (Calculated value)	BUN/CRE-S	None
Glucose	GLU	mg/dL
Albumin	ALB	g/dL
Total protein	TPRO	g/dL
Bilirubin, Total	T BILI	mg/dL
Globulin*	GLOB	g/dL
A/G Ratio*	A/G	-

[a] Abbreviations from the chemistry system readout may differ from those listed above, but the results will be reported as described above.*If there is not enough serum sample these parameters will not be assessed.

Shipping Blood will be shipped from UCLA to Quality Veterinary Laboratory for processing.

Joan Shewmaker CLS, MT(ASCP)
Manager
Quality Veterinary Laboratory
Telephone No.: 530-759-8533
Fax: 530-759-8553
E-mail: joan@qualityvetlab.com
Website: www.qualityvetlab.com

The Principal Investigator for hematology and serum chemistry will be responsible for all delegated phase activities and will provide hematology and serum chemistry data to the Study Director to send to the anatomic pathologist for interpretation.

15.11 Leukocyte Isolation from Spleen and Bone Marrow

Single cell suspensions will be prepared from spleen and from femurs and tibias of all mice for ddPCR and flow cytometry if needed (Table 4). Cells will be counted using an automated or manual cell counter (hemocytometer), and then DNA will be isolated from 1-5 x 10^6 cells for ddPCR. The remaining cells will be used for flow cytometry.

15.12 ddPCR

DNA will be extracted from blood, spleen and bone marrow following harvest. ddPCR analysis will be used to determine engraftment and persistence using primers that specifically amplify retroviral and lentiviral elements.

15.13 Flow Cytometry

Bone marrow isolated from femur and tibia and splenocytes isolated from spleens of recipient mice (at 3 months after injection) will be counted using an automated or manual cell counter. Expression of NY-ESO-1 TCR in both cell preparations will be determined by the intracellular staining of the beta chain of the NY-ESO-1 TCR (Vb13). Stem cell subsets will be characterized by surface staining of Lineage markers, Sca-1, cKit, CD48 and CD150 in bone marrow samples. T cell, B cell and granulocyte subsets will be assessed by surface staining of CD3, CD4, CD8, NK1, IgM, B220, Gr-1, CD-11b and F4/80 in splenocytes. Also to confirm the transgenic mouse phenotype, surface HLA-A2 staining will be performed on splenocytes from each mouse.

16.0 STATISTICAL ANALYSES

Descriptive statistics including mean, standard deviation, frequency, percentage and range will be calculated for quantitative endpoints such as retrovirus/lentivirus VCN measured by ddPCR, cell populations characterized by flow cytometry, hematology parameters (Table 5), serum chemistry measurements (Table 6) and animals' body and organ weights. Appropriate summary tables, scatter plots and box plots will be presented. Transformation for continuous endpoints will be performed as necessary to satisfy normality assumption before testing. Also, nonparametric approaches will be investigated as possible alternatives.

- Primary statistical analyses are listed as follows:

1) For the evaluation of T cell engraftment, unpaired t test will be performed to compare retrovirus VCN between Cohort D and Cohort E in blood and spleen harvested on Day 5 after transplant.
2) For the evaluation of HSC engraftment, unpaired t test will be performed to compare lentivirus VCN between Cohort C and Cohort E in bone marrow, blood and spleen at 3 months after transplant.
3) For the evaluation of the effect of transduced Lin- and T cell co-administration on HSC differentiation, unpaired t test will be performed to compare CBC parameters (e.g. RBC, WBC, platelets and Hemoglobin) between Cohort C and Cohort E at 3 months after transplant.

- Secondary statistical analyses are listed as follows:

1) For the evaluation of survival of transplanted animals, Kaplan-Meier survival curves will be plotted for each cohort and log-rank test will be used to compare the survival distributions between experimental cohorts.
2) For the evaluation of T cell persistence, unpaired t test will be performed to compare retrovirus VCN between Cohort D and Cohort E in blood and spleen harvested at 3 months after transplant.
3) For the evaluation of HSC differentiation, unpaired t test will be performed to compare the frequency of the different cell subsets (T cell, B cell and granulocyte) in the Vb13+ compartment compared to the whole population in the spleen between Cohort C and Cohort E at 3 months after transplant.
4) For the evaluation of HSC engraftment, unpaired t test will be performed to compare the TCR expressing (Vb13+) cells in the bone marrow between Cohort C and Cohort E at 3 months after transplant.

An alternative approach for analyzing the aforementioned primary and secondary VCN-related endpoints would be to dichotomize the VCNs (e.g. engraftment or not, persistence or not) based on the detection limit and Fisher's exact test will be used to compare the proportion of engraftment/persistence between cohorts.

- As exploratory statistical analyses, multi-factor ANOVA will be used to compare between experimental groups while adjusting for gender and possible group*gender interaction. To further adjust variations due to individual mice, cages and experiments, a multivariate linear mixed model approach will be adopted to include individual mice/cages/experiments as random effects

All statistical analyses will be carried out using SAS version 9.4 (SAS Institute Inc. 2013). Analysis results will be included as an appendix to the final report.

17.0 RECORDS, TEST ARTICLES AND SPECIMENS TO BE RETAINED

Specimens will be identified by study protocol number, experiment number, animal number, nature (i.e DNA, blood, etc.) and date of collection. Test and control articles will be identified with the protocol number, the experiment, the cell type, transduction status and the date. All test articles, controls and specimens will be archived by the sponsor at UCLA for 5 years after IND submission. All raw data and records that would be required to reconstruct the study will be maintained by the sponsor at UCLA Ribas Lab archive according to Ribas Lab SOP for at least 5 years after IND submission.

Records retained will include but not be limited to:

- Study protocols, SOPs, communication logs, completed forms or attachments and all raw data.

- Personnel records, job descriptions, CVs and training records.
- Test and control materials
    - Lentiviral and retroviral vector receipt, storage, usage and distribution
    - Lineage depleted cell generation, storage, usage and distribution
    - T cell generation, storage, usage and distribution
- Facilities
    - Animal room temperature, humidity and light logs
    - Water, feed and bedding analysis
    - Equipment calibration, and maintenance
    - Cold and warm storage records
- Cell culture and transduction
    - Cell viability prior to transplant
    - T cell and Lin- cell purity
    - Results of ddPCR-evaluation of transduction
    - Sterility, mycoplasma, endotoxin test and VCN
- In-life phase
    - Animal transfer and disposition
    - Pre-dosing observations and health assessment
    - Animal randomization and identification
    - Body weights, clinical observations and health checks
    - Transplantation records
    - Records of antibiotic treatment delivered to animals
    - Sample collection and hematology/serology results from moribund mice
- Endpoint data and analyses
    - Blood collection
    - Hematology and serum chemistry
    - DNA extraction and quantity
    - ddPCR results and analyses
    - Flow cytometry
    - Necropsy records
    - Histopathologic analysis records

Test articles, viral vectors and specimens retained will include but not be limited to:

- Test articles and test controls (Reserve samples, see section 9.0):
    - Lin- cells transduced with LV-NY-ESO-1 TCR/sr39TK, at least 2 aliquots stored in Liquid Nitrogen (test article 1).
    - Lin- cells mock transduced , at least 2 aliquots stored in Liquid Nitrogen (control article).
    - T cells transduced with RV-NY-ESO-1 TCR, at least 2 aliquots stored in Liquid Nitrogen (test article 2).
    - T cells mock transduced, at least 2 aliquots stored in Liquid Nitrogen (control article)
- Viral vectors at least 5 aliquots of each.

- Blood smears, slides, blocks (with paraffin-embedded tissues) and fixed carcasses.
- All DNA samples.

Reserve samples will be archived no later than 30 days following the completion of the study with the submission of the final report.

18.0 FINAL REPORT

A complete report of the work performed will be audited by the Quality Assurance Unit (QAU) at UCLA. A comprehensive final report that encompasses interpretation of the results, statistics and all other work performed in support of this study will be prepared. The final draft report will be generated electronically. In addition, the original signed hard copies of the final report including all appendices will be generated and archived.

19.0 QUALITY ASSURANCE

Quality Assurance Unit (QAU) Audits

The QAU at UCLA will audit and issue a Quality Assurance Statement that will include all critical phases of the study including: initiation, in-life, pathology, reporting, and the final report. The QAU will make critical phase inspections and examine the data to ensure that it is complete, consistent and well documented. The QAU in conjunction with the Study director and responsible scientist(s) will formally examine the final report to ensure that it accurately represents the raw data collected in the study in accordance with Standard Operating Procedures/Protocols, Study Specific Procedures, the Study Protocol and as required by Good Laboratory Practice regulations (as applicable).

The QAU at the test sites (Histo Tox Labs, Inc,, and Quality Vet Lab) will be responsible for quality assurance at their respective facilities to comply with GLP regulations and a Quality Assurance Statement will be issued and included in the histopathology report.

20.0 AMENDMENTS AND DEVIATIONS

Changes to the approved protocol will be made in the form of amendments which will be signed and dated by the Study Director with Sponsor approval. Every reasonable effort will be made to discuss any necessary changes in advance with the Sponsor.

Deviations from Good Laboratory Practices, the Study Protocol, Study Specific Procedures/Protocols and Standard Operating Procedures will be promptly reported to the Study Director and Sponsor to assess the impact on the quality and integrity of the study. The deviations will be logged according to the appropriate Ribas lab SOP and documented on a deviation log sheet. The nature of the deviation, the effect on the study and the corrective action taken, if any, will be recorded in a Deviations log which will be included with the Final Report.

21.0 REFERENCES

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5. CMC section IND 17471

Module 3.2.P. Cell Product

This section has been written following FDA guidelines for gene therapy as described in the Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs), April 2008.

(A) PRODUCT MANUFACTURING-COMPONENTS AND MATERIALS

1. VECTORS: Gene Therapy Vector Constructs

1.1 Retroviral Vector MSGV1-A2aB-1G4A-LY3H10

The MSGV1-A2aB-1G4A-LY3H10 retroviral vector encodes for the NY-ESO-1 T cell receptor (TCR). Manufacturing information regarding this product can be found in IND 15167 (Sponsor: Ribas, UCLA), entitled "Adoptive Transfer of NY-ESO-1 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) after a Nonmyeloablative Conditioning Regimen, with Administration of NY-ESO-$1_{157\text{-}165}$ Pulsed Dendritic Cells and Interleukin-2, in Patients with Advanced Malignancies". The Certificate of Analysis (CoA) for the MSGV1-A2aB-1G4A-LY3H10 retroviral vector can be found in Module 3.2.A, Appendix # 3.

1.2 Lentiviral Vector LV-optNYESO TCR/sr39 TK

1.2.1. Gene Therapy Vector Construct

The immunotherapeutic lentiviral vector LV-optNYESO TCR/sr39TK will be used to transduce peripheral blood stem cells (PBSCs) allowing progeny cytotoxic T lymphocytes (CTLs) to specifically recognize NY-ESO-1-positive HLA-A2.1-positive tumor cells. The integrated lentiviral vector LV-optNYESOTCR/TK consists of 9417 bps including the 3rd generation lentiviral self-inactivating (SIN) long terminal repeats (LTRs), internal promoter from the murine stem cell virus (MSCV), packaging signal with the splicing donor and splicing acceptor sites, alpha chain and beta chain genes of the NY-ESO-1 TCR from Tumor Infiltrating Lymphocyte (TIL) clone 1G4 α95:LY, and the positron emission tomography (PET) reporter/suicide gene sr39 thymidine kinase (TK). The alpha and beta TCR chains and the sr39TK genes are linked by 2A self-cleaving sequences (P2A and T2A, respectively). **Figure 1** below shows a schematic of the integrated proviral part of this vector. The plasmid pRRL-MSCV-opt NYESO-optsr39TK-WPRE-1 that was used to produce the LV-optNYESO TCR/sr39TK vector was generated in the Ribas laboratory at UCLA, and both were manufactured at clinical grade at the Indiana University Vector Production Facility (IUVPF). Lentiviral stability studies were performed showing stability was not impacted within an 11 month period (Module 3.2.A, Appendix # 6).

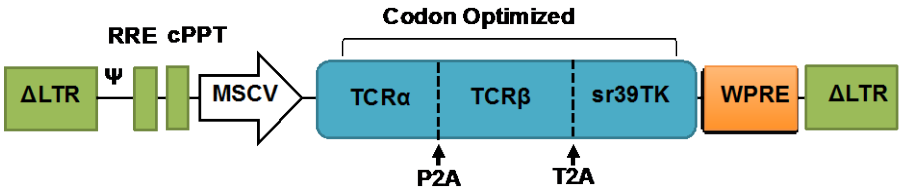
The pRRL-MSCV-optNYESO optsr39TK-WPRE-1 is a self-inactivated 3rd generation lentiviral transfer vector containing chimeric Rous sarcoma virus (RSV)- 5′ LTRs. The vector contains a U3 promoter region of MSCV that drives the expression of the TCR and a WPRE (woodchuck hepatitis virus posttranslational response element) sequence that enhances the expression of the transgene, vector stability and titer.

The vector also contains the human immunodeficiency virus-1 (HIV-1) central polypurine tract (cPPT) that is important for nuclear import of the vector DNA and the Rev response element (RRE) that is essential for post-transcriptional transport of the unspliced and incompletely spliced viral mRNAs from the nucleus to the cytoplasm. The packaging signal directs the incorporation of the vector RNA into the virions. The LTRs of the vector contain the signals required for reverse transcription. The 3 proteins (TCR α chain, TCR β chain and sr39TK) are expressed through the same promoter fused with the self-cleaving 2A sequences. The TCR α and β chains are linked with a furin cleavage site (RAKR) followed by an SGSG spacer and P2A peptide (derived from porcine teschovirus-1) and the sr39TK is fused to the TCR β through a T2A (from Thoseaasigna virus).

1.2.2 Vector Diagram.

Please refer to **Figure 1** below.

Figure 1. LV-optNYESOTCR/TK lentiviral vector provirus.



1.2.2 Sequence Analysis

The vector sequence has been verified by transducing peripheral blood CD34+ cells with clinical grade LV-optNYESO TCR/sr39TK vector lot# 111015L12. The CD34+ cells were harvested 14 days after transduction and DNA was extracted for sequencing. The sequence obtained was compared to the reference sequence obtained from the vector plasmid and no differences were seen. The full sequence of the vector and sequence analysis is reported in Module 3.2.A, Appendix # 2.

2. CELLS

2.1 PBMC Transgenic for NY-ESO-1 TCR (RV-NYESO TCR PBMC)

2.1.1 Cell Source

The cell source is autologous PBMC from patients with advanced malignancies which will be collected by unmobilized leukapheresis at the UCLA Hemapheresis Unit.

2.1.2 Collection or Recovery Method

Patients will undergo an approximate 6-liter leukapheresis without prior mobilization to obtain PBMC for retroviral transduction. The leukapheresis product will be collected into sterile bags at the unit. The leukapheresis product will be transported to the cell-processing laboratory at the UCLA GMP facility. PBMC obtained from leukapheresis will be genetically modified with the clinical grade MSGV1-A2aB-1G4A-LY3H10 retroviral vector and infused fresh to patients after the lot release tests are completed.

Additional manufacturing information about this product can be found in IND 15167 (Sponsor: Ribas, UCLA), entitled “Adoptive Transfer of NY-ESO-1 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) after a Nonmyeloablative Conditioning Regimen, with Administration of NY-ESO-$1_{157-165}$ Pulsed Dendritic Cells and Interleukin-2, in Patients with Advanced Malignancies” (also, see Module 5.3, Clinical Protocol, Section 10).

2.1.3 Donor Screening and Testing

Although these cells are for autologous use, as part of the screening process for inclusion into the study, subjects will be excluded from participation in the clinical trial if they have evidence of Human Immunodeficiency Virus (HIV), cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) or Herpes Simplex Virus-1 (HSV-1).

2.2 CD34+ PBSC Transgenic for NY-ESO-1 TCR/sr39TK (LV-NYESO TCR/sr39TK PBSC)

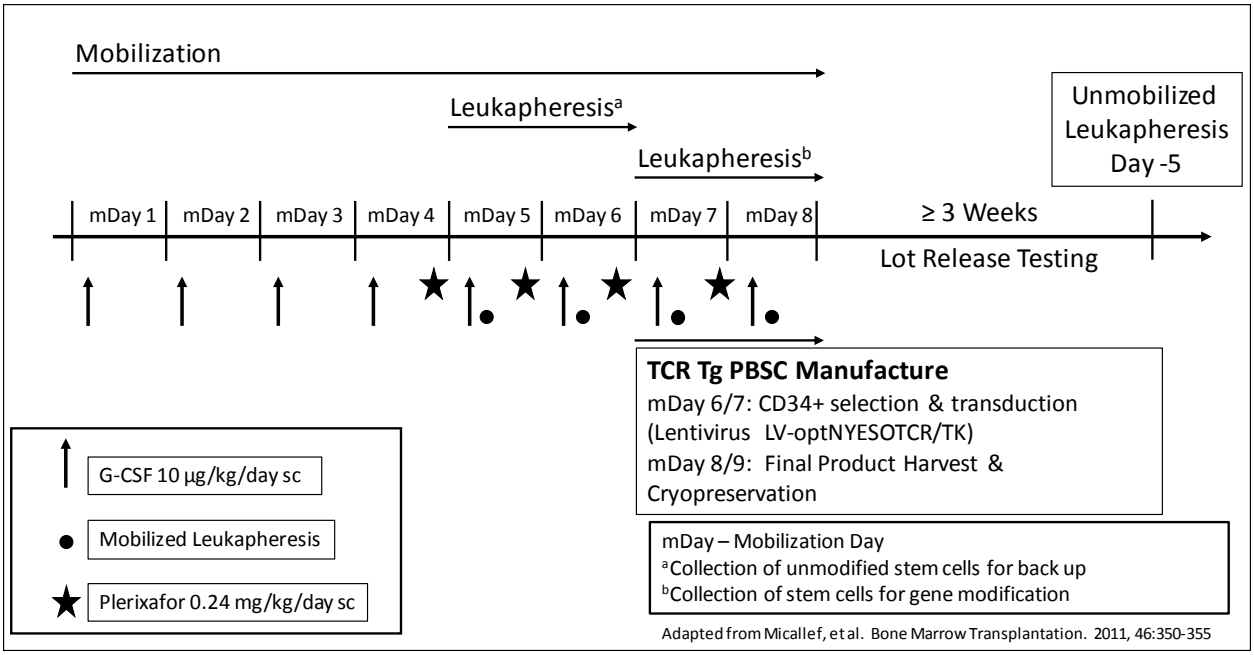
2.2.1 Cell Source

The cell source is autologous PBSC from patients with advanced malignancies, which will be collected by mobilized leukapheresis at the UCLA Hemapheresis Unit.

2.2.2 Mobilization protocol

PBSC will be mobilized by administration of G-CSF and plerixafor. Six months to 3 weeks prior to infusion of transduced cells, patients will undergo G-CSF and plerixafor mobilization of PBSC, which will be obtained by leukapheresis (Module 5.3, Clinical Protocol, Section 8.3). The mobilization protocol is summarized in **Figure 2** below.

Figure 2. Stem cell mobilization and manufacture scheme



Patients will receive (or self-administer) G-CSF (10 µg/kg/day) subcutaneously on mobilization days (mDay) 1-4 in the morning.

- On the evening of mDay 4, patients will begin receiving plerixafor (0.24 mg/kg/day subcutaneously).
- Daily administration of G-CSF in the morning and plerixafor in the evening will continue until the target number of CD34+ cells and cells for backup are collected up to mDay 8. A sufficient number of un-manipulated cells are required for backup in case of engraftment failure.

2.2.3 Collection or recovery method

- A 12-liter leukapheresis will begin approximately 10-11 hours after administration of plerixafor in the morning of mDay 5. Daily leukaphereses will continue until a sufficient number of cells for backup and transduction are collected up to mDay 8.
- At least $2x10^6$ CD34+ un-manipulated cells/kg (without CD34+ cell isolation) have to be obtained on mDay 5 and/or mDay 6 in order to proceed to collection of cells for transduction. Backup cells will be cryopreserved in liquid nitrogen vapors (≤ -140°C) in the UCLA Bone Marrow/Stem Cell Transplant Laboratory for possible future use if there is lack of engraftment (**Figure 2**) according to SOP 1023.02.
- If enough cells are obtained for back up on mDay 5/6, mobilized cells will then be collected for transduction on mDay 6/7 through mDay 7/8 (depending on how many days it takes to collect backup cells) over a maximum of 2 days.
- If a sufficient number of cells are obtained (≥$2.5x10^6$ nucleated cells/kg, after CD34 enrichment), CD34+ PBSC will be stimulated, transduced with the LV-optNYESOTCR/TK lentiviral vector and cryopreserved until ready for use after lot release testing. The number of transduced CD34+ cells needed is 2-$6x10^6$ cells/kg (Module 5.3, Clinical Protocol, Section 10).

2.2.4 Donor screening and testing

Although these cells are for autologous use, as part of the screening process for inclusion into the study, subjects will be excluded from participation in the clinical trial if they have evidence of Human Immunodeficiency Virus (HIV), cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) or Herpes Simplex Virus-1 (HSV-1).

3. REAGENTS

3.1 Tabulation of Reagents Used in Manufacture

Table 1. Reagents used in cell product manufacturing process

RV-NYESO TCR PBMC##					
Reagent	**Vendor/Supplier**	**Final concentration**	**Use**	**Source**	**Grade**
PBMC Isolation					
Ficoll-Paque PLUS	GE Healthcare	(×1)	Density gradient	N/A	Research *
0.9% Sodium Chloride (normal saline)	Baxter	(×1)	Buffered medium for PBMC collection	N/A	USP*

Cell culture and transduction					
AIM-V® Medium CTS TM Gentamicin Sulfate [-] Streptomycin Sulfate [-]	Life Technologies Custom manufactured	(×1)	Culture Media		GMP*
Human AB Serum	Omega Scientific, Inc.	5% in AIM-V	Component in culture media	Human	Research *
IL-2	Boehringer Ingelheim	300 IU/ml	Component in culture media	E. Coli	USP*
OKT-3 (anti-human CD3 antibody)	Miltenyi Biotec	50 ng/ml	Component in stimulation media	Mouse	GMP*
PBS	Lonza	(×1)	Used to dilute Retronectin	N/A	Research *
HBSS	Lonza	(×1)	Component in Retronectin washing buffer	N/A	Research *
Hepes	Lonza	(×1)	Component in Retronectin washing buffer	N/A	Research *
Recombinant human fibronectin fragment (Retronectin)	Takara/Clontech	10µg/ml	Facilitates lentiviral gene transduction	N/A	GMP*
RV-NYESO-TCR vector	IUVPF	N/A		N/A	GMP*★
Final formulation					
0.9% Sodium Chloride (normal saline)	Baxter	(×1)	Component of final formulation media	N/A	USP*
Albumin (Human) U.S.P., 25%	Grifols	1% in normal saline	Component of final formulation media	Human	USP*

LV-NYESO TCR/sr39TK PBSC					
Reagent	**Vendor/Supplier**	**Final concentration**	**Use**	**Source**	**Grade**
CD34 isolation					
CliniMACS PBS/EDTA Buffer	MACS Miltenyi Biotec	(×1)	Cell wash	N/A	Clinical*
IVIG (Gammagard)	Baxter International Inc.	5% prior CD34 selection	Blocking prior to CD34 selection	Human	USP*
CliniMACS® CD34 Reagent	Miltenyi Biotec	7.5ml/6x10^6 cells	CD34 selection	N/A	Clinical*#
Albumin (Human) 25%	Grifols	0.5% in PBS/EDTA buffer	Component of the washing PBS/EDTA buffer	Human	USP*
Cell culture and transduction					
X-Vivo 15	Lonza	(×1)	Culture media	N/A	Research *
MACS GMP Recombinant Human SCF	MACS Miltenyi Biotec	50 ng/ml	Supports hematopoietic culture expansion	E. Coli	GMP*

MACS GMP Recombinant Flt3-Ligand	MACS Miltenyi Biotec	50/ng/ml	Supports hematopoietic culture expansion	E. Coli	GMP*
MACS GMP Recombinant Human TPO	MACS Miltenyi Biotec	50 ng/ml	Supports hematopoietic culture expansion	E. Coli	GMP*
MACS GMP Recombinant Human IL-3	Miltenyi Biotec	20 ng/ml	Supports hematopoietic culture expansion	E. Coli	GMP*
Recombinant human fibronectin fragment (Retronectin)	Takara/Clontech	4µg/cm^2	Facilitates lentiviral gene transduction	N/A	GMP*
DPBS	Life Technologies	(×1)	Washing buffer	N/A	Research *
Hanks' BSS (HBSS)	Lonza	(×1)	Washing buffer	N/A	Research *
Albumin (Human) U.S.P., 25%	Grifols	0.5% in PBS/EDTA buffer	Washing buffer 1% in HBSS Blocking buffer 2% in DPBS	Human	USP*
Sterile Water for Injection	Hospira Healthcare	(×1)	Cytokine and Retronectin reconstitution	N/A	USP*
LV-optNYESO TCR/sr39TK	IUVPF	50 MOI		Human	GMP*[#]
Cryopreservation and final formulation					
Pentastarch Solution	Preservation Solutions	(×1)	Component of the cryopreservation media	N/A	USP*
Plasma-Lyte	Baxter	(×1)	Component of cryopreservation media	N/A	USP*

Cryoserv	Mylan Institutional	5% in cryopreservation media	Component of cryopreservation media	N/A	USP*
Albumin (Human) U.S.P., 25%	Grifols	5% in cryopreservation media	Component of cryopreservation media	Human	USP*

*Example Certificate of Analysis attached for each reagent (Module 3.2.A Appendix # 3)
Refer to corresponding cross-reference letter (Module 1.4, Appendix # 4)
⋆ Refer to cross-reference letter as in IND 15167 (Module 1.4, Appendix # 2)
This is an updated list of the reagents for RV-NYESO TCR PBMC manufacturing; it has also been recently been updated in IND 15167, it includes updated vendors and reagent grades.

3.2 Qualification Program

Research-grade reagents used in manufacturing will be assessed based on the vendor's Certificates of Analysis. If according to the manufacturer's CoA, a reagent was not assessed for safety (sterility, endotoxin, mycoplasma, adventitious agent), we will test an aliquot for sterility (CLIA Certified Microbiology Laboratories) and endotoxin (EndoSafe®-PTS^{TM}). Testing for mycoplasma and adventitious agents will not be performed unless warranted (i.e. production of the reagent involved cell culture or the preparation of the reagent involved animal products). This process will be repeated each time a new lot of reagent is used.

3.3 Removal of reagents from final product

After culture, the cells will be washed in a buffer consisting of HBSS + 1% HSA (LV-NYESO TCR/sr39TK PBSC) or normal saline (RV-NYESO TCR PBMC) for a minimum of three times before the final formulation.

3.4 Other concerns

Antibiotics are not used during the manufacture of the therapeutic product.

3.5 Excipients

The final product will be formulated in normal saline supplemented with 1%HSA (RV-NYESO TCR PBMC) or in a cryopreservation solution consisting of Plasmalyte-A and DMSO (Cryoserv) (5%), Pentastarch (6%), HSA (5%).

(B) PRODUCT MANUFACTURING-PROCEDURES

1. VECTOR PRODUCTION/PURIFICATION

1.1. Retroviral vector RV-NYESO TCR

The vector was manufactured at IUVPF following FDA Good Manufacturing Practice (GMP) guidelines. Please refer to IUVPF's letter authorizing cross-reference to BB MF 8653 (Module 1.4, Appendix # 2) issued for IND 15167, entitled "Adoptive Transfer of NY-ESO-1 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) after a Nonmyeloablative Conditioning Regimen, with Administration of NY-ESO-$1_{157-165}$ Pulsed Dendritic Cells and Interleukin-2, in Patients with Advanced Malignancies".

1.2. Lentiviral Vector (LV-NYESO TCR/sr39TK)

The vector was produced at IUVPF following FDA GMP guidelines. Please see authorization letter from IUVPF to cross-reference BB MF 10640 (Module 1.4, Appendix # 3).

2. PREPARATION OF *EX VIVO* GENE-MODIFIED AUTOLOGOUS CELLS

2.1. RV-NYESO TCR PBMC

PBMC obtained from leukapheresis will be activated for 48 hours with OKT3 (anti-CD3 antibody) and IL-2 and then genetically modified with the clinical grade MSGV1-A2aB-1G4A-LY3H10 retroviral vector supernatant for two consecutive days. These cells will be maintained in culture for 4 days from the start of transduction in media supplemented with IL-2 and then are infused fresh to patients. Aliquots of these cells will be used to fulfill the lot release criteria.

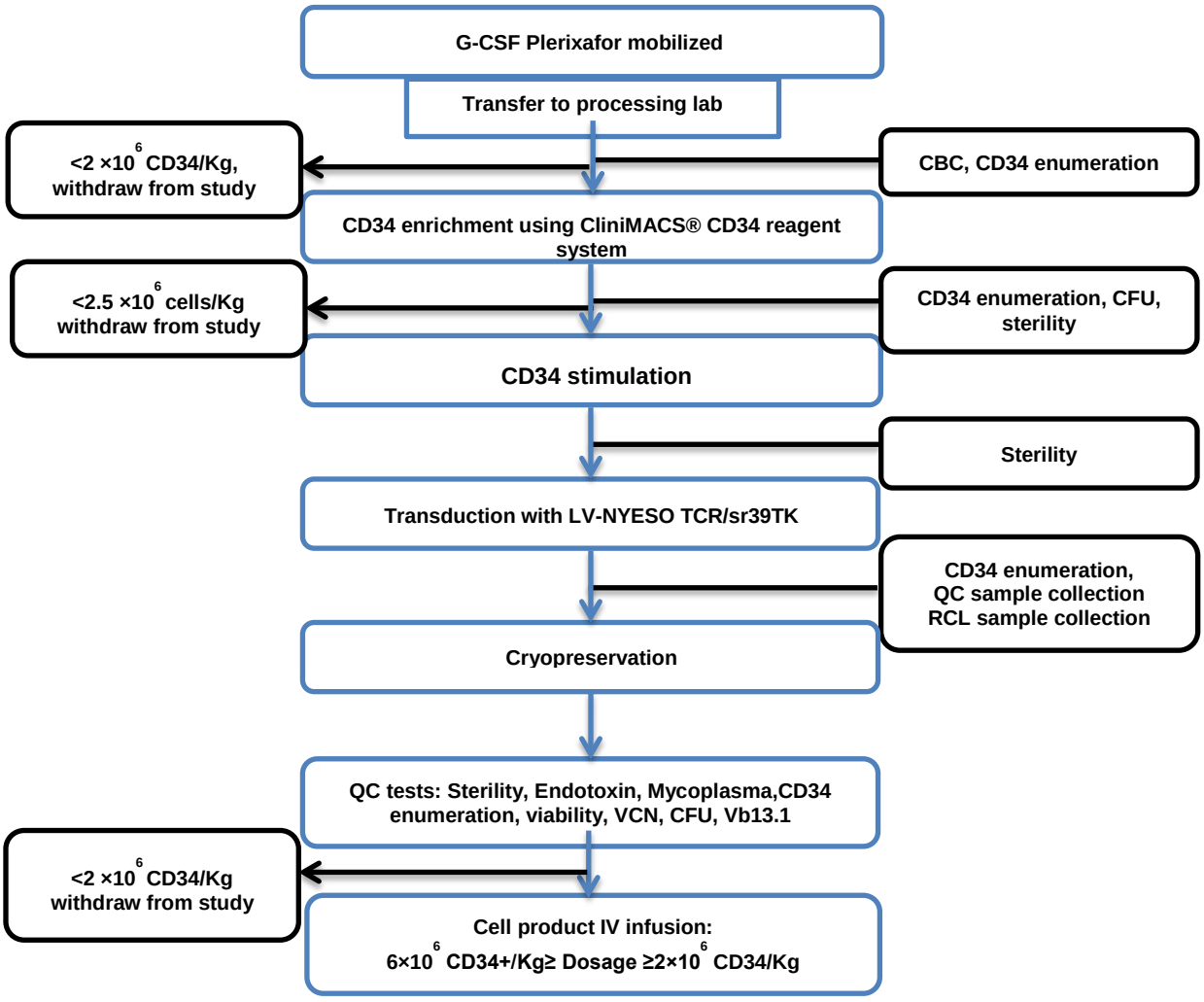
Additional manufacturing information about this product can be found in IND 15167 (Sponsor Ribas, UCLA), entitled "Adoptive Transfer of NY-ESO-1 TCR Engineered Peripheral Blood Mononuclear Cells (PBMC) after a Nonmyeloablative Conditioning Regimen, with Administration of NY-ESO-$1_{157-165}$ Pulsed Dendritic Cells and Interleukin-2, in Patients with Advanced Malignancies" (also, see Module 5.3, Clinical Protocol, Section 10).

2.2 LV-NYESO TCR/sr39TK PBSC

2.2.1 Cell Collection/Processing/Culture

The overview of the manufacturing procedure is summarized in **Figure 3** below.

Figure 3. Product manufacturing scheme



Cells will be collected from the subject's mobilized peripheral blood by leukapheresis at the UCLA Hemapheresis Unit and transported to the cell-processing laboratory at the UCLA GMP facility. A sample will be submitted for CBC and CD34+ cell enumeration (ISCHAGE flow cytometric method). The initial fresh leukapheresis product will be washed to decrease platelet content. The CD34+ cell population will be enriched using CliniMACS®CD34 reagent system, which consists of CliniMACS® Plus Instrument, CliniMACS® single use, sterile, disposable tubing set and CliniMACS® PBS/EDTA buffer following a standard manufacturing procedure (SOP) developed based on the manufacturer's instructions. The resulting cell fraction will be characterized for total cell number, viability, and percentage of CD34+ cells.

2.2.2 Acceptance criteria

- If the resulting CD34+ cell number is ≥2.5 x10^6 nucleated cells/kg, after CD34 enrichment, the cells will be processed for gene modification. If the resulting nucleated cell number is <2.5 x10^6 cells/kg the subject will be withdrawn from further study participation.
- If sufficient CD34+ cells are obtained from the CliniMACS® and the procedure continues, additional testing on the CD34+ fraction will include a CFU assay in methylcellulose and sterility.

2.2.3 Pre-stimulation (Day 0 i.e. mDay 6/7 – see **Figure 2**)

The cells will be cultured at a concentration of 1x10^6 cells/ml on Retronectin coated surfaces and pre-stimulated for 18 ± 6 hours (~37° C, 5% CO_2) in serum-free X-VIVO15 medium supplemented with the following cocktail of cytokines: rhSCF (50ng/ml), Flt-3 ligand (50ng/ml), TPO (50 ng/ml) and IL-3 20ng/ml. An in-process sterility sample will be collected and submitted for analysis.

2.2.4 Transduction (Day 1 i.e. mDay 7/8 – see **Figure 2**)

After the pre-stimulation period, non-adherent cells will be removed from the culture flasks and spun down in sterile 250ml centrifuge tubes. An aliquot of supernatant will be sampled and submitted for sterility testing. The non-adherent cells will be resuspended in fresh cytokine-containing medium. The resuspended non-adherent cells will be added back to the original flasks containing the adherent cell fraction at an approximate cell density of 2-3 x10^6 cells/ml. The LV-NYESO sr39TK vector will be added at a final multiplicity of infection (MOI) of 50 (50 TU/cell) performed in two rounds of transduction of 25 MOI each. The total transduction period is 18 ± 6 hours (~37° C, 5% CO_2). In-process test results prior to final product harvest are summarized in Table 2.

Table 2. Summary of In-Process Test Results

Sample description	**Test Description**	**Method**	**Specification for Intermediate Acceptance**
Mobilized Leukapheresis	CD34+ enumeration	ISHAGE	≥2×10^6 cells/kg

	CBC	Automated cell counter	Report results
Post-CliniMACS Positive fraction	CD34+ enumeration	ISHAGE	≥2.5×10^6 nucleated cells/kg
	CFU potential	Methylcellulose culture	Report results
	VCN	ddPCR	Report results
	Sterility culture		Negative at the day of cryopreservation
18±Post stimulation	Sterility culture		Negative at the day of cryopreservation
18±Post transduction	CD34+ enumeration	ISHAGE	≥2×10^6 cells/kg
	RCL supernatant		Banked

2.2.5 Cell product harvest (Day 2 i.e. mDay 8/9)

After 36 ± 12 hours from the initiation of culture, the entire cell culture (consisting of adherent and non-adherent cells) will be harvested for formulation and infusion. The adherent cells will be collected by washing with HBSS/1%HSA. The adherent and non-adherent cell suspensions will be centrifuged. Following centrifugation, a portion of the supernatant from each tube will be removed, pooled together and banked (frozen) for replication competent lentivirus (RCL) testing. RCL testing will be performed only if warranted (i.e. RCL is detected in the subject's peripheral blood during the follow-up period).

2.2.6 Cell product formulation

The cells will be washed 3 times in wash buffer (HBSS + 1% HSA) and then resuspended and formulated in cryopreservation solution consisting of Plasmalyte-A/DMSO (Cryoserv) (5%)/Pentastarch (6%)/HSA (5% at a concentration of 1.5-40×10^6 cells/ml). All the reagents used for final formulation are clinical grade. The formulated cell suspension will be filled into cryobags. The final formulation volume will acount for the QC tests, RCL (1% of the final product) and reserve sample/s that will be collected from the cell product container immediately following the fill and cryopreserved separately in sterile cryovials. Cell product, RCL, reserved and QC samples will be cryopreserved using a controlled-rate freezer at the same time under the same conditions. Upon completion of the controlled freeze, the cell product cryobags and QC samples will be transferred to storage in the vapor phase of LN2. Cryobags with cell product will be stored in the vapor phase of LN2 until released and required for administration into the subject. The cryopreserved QC sample will be thawed and subjected to lot release testing as summerized in Table 3.

Table 3: Final lot release criteria

Test	**Method**	**Acceptance Criteria**	**Performed at**
CD34 enumeration	ISHAGE flow cytometric method	≥2.5×10^6 nucleated cells/kg, after CD34 enrichment	CTL
% CD34 purity	ISHAGE flow cytometric method	≥50%	CTL
Cell viability	Trypan Blue manual count	≥70%	In House
Vector copy number	ddPCR	0.1-2 copies/cell	In House
Sterility	Sterility culture	Negative	CTL
	Fungal culture	Negative	CTL
Endotoxin	Endosafe®PTS	≤ 5 EU/Kg	In House
Mycoplasma	MycoAlert test	Ratio<1	In House
CFU assay	CFU potential Methylcellulose	≥10%	In House
% LV-NYESO+ CFUs	ddPCR	5%-50% LV-NYESO+ colonies*	In House
Vβ13.1[#] expression	Flow Cytometry	5%-50% of Vb13.1+ cells*	In House

CTL- Contract Testing Laboratory

[#]Vb13.1 is TCR β chain variant of NY-ESO-1 TCR

* In order to make sure that the repertoire of endogenous TCRs will not be compromised, at least 50% of the CD34+ PBSC delivered to the patient will be untransduced.

The cryopreserved gene-modified cells will be thawed and infused only after the product manufacturing batch record has been reviewed and the product has been released by quality assurance personnel. The number of the infused cells will be limited to ≤ 6 x 10^6 cells/kg. If the number of cells harvested after culture exceeds this limit, the remaining cells will be discarded or released for research.

Stability studies of the cryopreserved final cell product have been conducted. Preliminary stability results show that the cell product stored in the vapor phase of LN2 is stable for up to 6 months post-cryopreservation and for up to 24 h at 4-8°C post-thaw, the results have been included in the final cell product stability report.

2.2.7 Administration of Cryopreserved Cell Product into the Subject

Cells will only be administered to patients at a single site at UCLA. The cells will not be shipped. For transport within the UCLA clinical site, cell product cryobag(s) will be placed into a temperature monitored dry nitrogen shipping container. For administration, the shipping container will be taken directly to the subject's bedside. Immediately prior to infusion, cell product cryobag(s) will be removed from the shipping container and thawed using either a plasma thawing device or a pre-warmed water bath. Upon thaw, cell product bag(s) will immediately be infused

directly without further manipulation per Bone Marrow/Stem Cell Transplantation Unit's infusion SOP.

(C) OTHER

1. PRODUCT TRACKING

The product tracking will be performed per SOP # C1040, Product Tracking. In most cases, only one cell product will be manufactured at a time. In the event that more than one product is manufactured in a clean room, the surfaces and equipment will be thoroughly cleaned, all opened reagents and trash removed between procedures as part of the change control procedure as per GMP facility SOP# 420-Change Control Procedure.

2. PRODUCT LABELING

The product labeling will be performed per SOP# 1050, Cell product and related sample labeling. The examples of the labels for RV-NYESO TCR PBMC and LV-NYESO TCR/sr39TK PBSC cell products are shown in Figure 4.

FIGURE 4: Example of the label for LV-NYESO TCR/sr39TK PBSC cell product

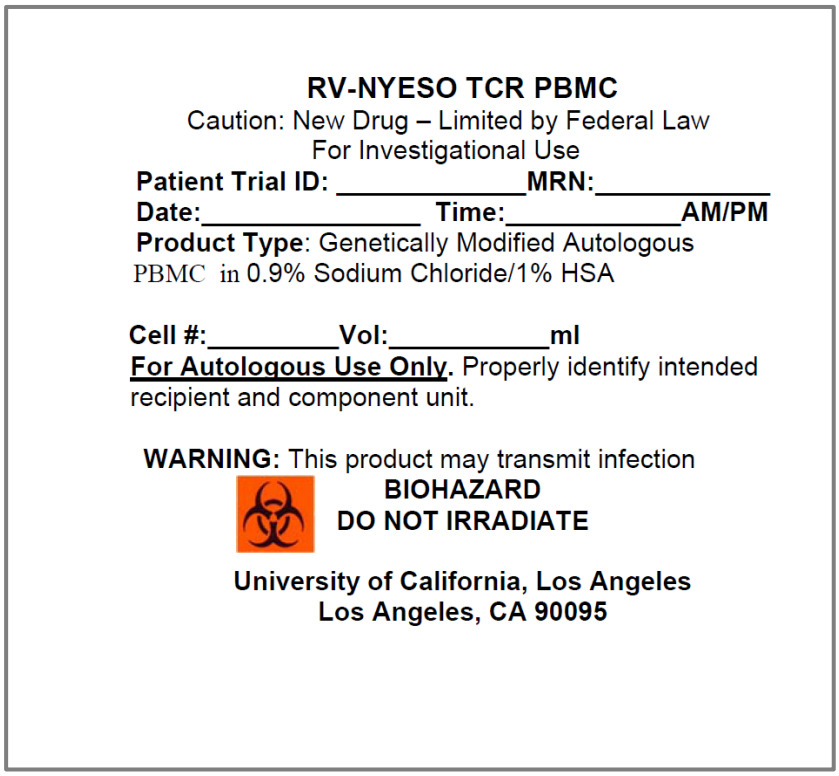
LV-NYESO TCR/sr39TK PBSC
Caution: New Drug – Limited by Federal Law
For Investigational Use
Patient Trial ID: _____MRN:_____
Date:_____ Time:_____AM/PM
Product Type: Genetically Modified Autologous
PB CD34+ Cells in Plasma-LyteA+5%DMSO+Pentastarch

CD34+ cell #:_____ Vol:_____ml
For Autologous Use Only. Properly identify intended recipient and component unit.
Checked by (initials): _____

WARNING: This product may transmit infection
BIOHAZARD
DO NOT IRRADIATE

University of California, Los Angeles
Los Angeles, CA 90095

Figure 4 Continued



3. CONTAINER/CLOSURE

The final product container is a CryoStore™ CS50 Cryobag (OriGen biomedical) which is a Class I 510(k) cleared medical device (pre-market submission number BK030036).

4. ENVIRONMENTAL IMPACT

We request a categorical exclusion from the requirements of preparing an environmental assessment. The drugs described in this IND are intended to be used for clinical studies and/or research programs in which waste disposal will be controlled, the amount of waste expected to enter the environment is reasonably expected to be nontoxic and in minimal quantities, and to the knowledge of the applicant, no extraordinary circumstances exist (21 CFR 25.15 (d)).

5. QUALIFICATION OF THE MANUFACTURING PROCESS

The autologous mobilized peripheral blood harvest and CD34+ isolation procedures will be performed per established SOPs.

The UCLA QC plan and a description of the UCLA GMP Manufacturing Suite is attached in Module 3.2.A, Appendix # 5. Specifically, the following functions are addressed:

- Examination of components used in the manufacturing process GMP facility SOP 1100, Receipt of Reagents & Supplies.
- Releasing or rejecting a clinical batch GMP facility SOP 1010, Product Release.
- Investigating and Initiating corrective action GMP facility SOP 440, Reporting deviations and GMP facility SOP 441, Corrective and Preventive Action Plans.
- Review and Approval of production procedures, testing procedures and acceptance criteria GMP facility SOP 490, Document control.

6. Stability report PBSC cell product IND 17471

APPROVALS:

Report Prepared by:_____ Date:_____

Beata Berent-Maoz, PhD

Statistical analysis:_____ Date:_____

Xiaoyan Wang, PhD

Reviewed by: _____ Date:_____

Antoni Ribas MD, PhD

Quality Assurance:_____Date:_____

Sujna Raval-Fernandes, PhD

TABLE OF CONTENT:

1. BACKGROUND

The stability of LV-NYESO TCR/sr39TK PBSC (also referred to as cell product) post-cryopreservation has been determined using the cryopreserved cell products from five healthy donors that were manufactured independently. The stability validation procedure was performed following SOP # 1610: Stability assessment of the cryopreserved LV-NYESO TCR sr39TK transduced autologous PBSC. The study was conducted per protocol AR-CIRM-17-002-P. As detailed herein, all acceptance criteria were either directly met, or any protocol discrepancies identified were investigated, justified and determined to have no impact on the qualification.

2. PURPOSE

To compare the attributes and stability of **Peripheral Blood Stem Cells (PBSC) transduced with LV-NYESO TCR/sr39TK** as a fresh formulation and cryopreserved in Cryoserv/Pentastarch cryopreservation media.

3. SCOPE

The current study compared the attributes of the fresh cell product with the cryopreserved cell product formulated in Cryoserv/Pentastarch cryopreservation media and assessed:

3.1. The long term stability of the cryopreserved cell product after 30 ±7 days (1 month), 90±7 days (3 months), 180±10 days (6 months) and 360±14 days (1 year) at ≤ -140°C (vapor phase of liquid nitrogen (LN2).

3.2. The short-term stability of the thawed cell product after 3, 6, 24 and 48 hours at ambient temperature and 4-8°C.

4. RESPONSIBILITY

Sponsor:	Antoni Ribas, MD, PhD University of California, Los Angeles
Test Facility:	Ribas Lab 14-638 Factor Bldg. UCLA Medical Center 10833 Le Conte Ave Los Angeles, CA 90095
Principal Investigator:	Antoni Ribas , MD, PhD University of California, Los Angeles
Study Coordinator:	Beata Berent-Maoz, PhD University of California, Los Angeles

5. ACCEPTANCE CRITERIA

Test	Method	Specification Fresh cell product	Specification Thawed cell product
CD34+ cell enumeration (cells/ml)	Flow Cytometry (ISHAGE Protocol)	Record results	Record results
% CD34+ cells (purity)	Flow Cytometry (ISHAGE Protocol)	≥50%	≥50%
%TNC viability	Trypan Blue manual count	≥70%	≥70%
% CD34+ cell recovery		Record results	Record results
Endotoxin[#]	Endosafe®PTS	≤ 5 EU/Kg	≤ 5 EU/Kg
Mycoplasma	MycoAlert test	Ratio<0.9	Ratio<0.9
Sterility	Sterility culture	No growth	No growth
	Fungal culture	No growth	No growth
%Vβ13.1 positive cells	Flow Cytometry	Record results	Record results
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	Record results
% LV-NYESO TCR/sr39TK positive CFU	ddPCR	Record results	Record results
Vector copy number (VCN)*	ddPCR and/or qPCR	Record results	Record results

* Analyses performed after cells are cultured for 14 days to avoid over estimating vector copy number (VCN) and potency due to the presence of un-integrated forms of the lentiviral vector.

[#] The endotoxin level was calculated based on the weight of the donor reported by a vendor.

6. REFERENCES AND APPLICABLE DOCUMENTS

SOP# C1610 - Stability assessment of the cryopreserved LV-NY-ESO1 TCR sr39TK transduced autologous CD34+cells.
SOP # C1000- Transduction of CD34+ cells with LV-NY-ESO 1 TCR sr39/TK.
SOP# C1601- CD34+ cell separation – CliniMACS Plus (One Leukapheresis Product)

7. HEALTH AND SAFETY CONSIDERATIONS

Refer to the Health and Safety Considerations section of the specific production batch records (PBRs), standard operating procedures (SOPs) and applicable manuals employed in this protocol, including those for the handling of compressed gases and LN_2.

8. DEFINITIONS

8.1. °C: Degree Centigrade
8.2. CFU: Colony Forming Unit
8.3. CO2: Carbon Dioxide
8.4. CRF: Controlled Rate Freezer
8.5. ddPCR: Droplet Digital Polymerase Chain Reaction
8.6. FCP: Fresh Cell Product
8.7. HD: Healthy Donor
8.8. HSC: Hematopoietic Stem Cell
8.9. LN2: Liquid Nitrogen
8.10. LV: Lentivirus
8.11. MBR: Manufacturing Batch Record
8.12. MOI: Multiplicity of Infection
8.13. PB: Peripheral Blood
8.14. PBSC: Peripheral Blood Stem Cells
8.15. qPCR: quantitative Polymerase Chain Reaction
8.16. SOP: Standard Operating Procedure
8.17. TCP: Thawed Cell Product
8.18. TE: Transduction Efficiency

8.19. TNC: Total Nucleated Cells

8.20. TU: Transduction Unit

8.21. VCN: Vector Copy Number

9. EQUIPMENT AND MATERIALS

9.1. Equipment, Reagents and Consumables

Refer to the SOP# C1610, for a comprehensive list of equipment, reagents and consumables.

9.2. Starting materials

9.2.1. LV-NYESO TCR/sr39TK

The pRRL-MSCV-optNYESO-optsr39TK-WPRE construct was developed in the research laboratory of Dr. Antoni Ribas at UCLA. The lentiviral vector was generated at the Indiana University Vector Production Facility (IUVPF; lot# 071013BSL12 for GMP comparable vector, CoA issued Sep 04, 2013; or lot# 111015L12 for clinical grade vector, CoA issued Sep9, 2016). The titers of the GMP comparable and clinical vector are 6.7 x10e8 and 2.0x10e8 Transducing Units (TU)/mL, respectively. The LV-NYESO TCR/sr39tk vector will be used to transfer a functional copy of the genes encoding NY-ESO-1 TCR and HSV1-sr39TK to CD34+ cells isolated from mobilized peripheral blood.

9.2.2. Human CD34+ cells

The cell product was manufactured from human CD34+ cells isolated from mobilized peripheral blood following the laboratory's SOPs.

9.2.3. Source of human cells

G-CSF mobilized peripheral blood from healthy donors.

10. PROCEDURES

10.1. Number of manufacturing runs

Five independent manufacturing runs from 5 healthy donors (HD1-HD5) were carried out and the stability of cell products made from these runs was assessed in this study.

10.2. LV-NYESO TCR/sr39TK PBSC cryopreservation

LV-NYESO TCR/sr39TK PBSC were manufactured from human CD34+ cells isolated from mobilized peripheral blood following the laboratory's SOP-C1601, and transduced following SOP # C1000. The LV-NYESO TCR/sr39TK PBSC product was harvested and formulated in cryopreservation solution consisting of Plasmalyte-A; (5%) DMSO (Cryoserv); (6%)/Pentastarch and (5%) (SOP# C1000). Aliquots of fresh cell product samples were collected for further product characterization. Cell product was divided into a number of cryopreservation bags based on cell availability and cryopreserved using a controlled-rate freezer and transferred to LN2 (≤-140°C) freezer (SOP C1000). The final volume of cell product was either 10 or 20 ml. The cell concentration in the cell product varied based on cell availability.

10.3. TCR/sr39TK PBSC stability evaluation

Stability of the cell product was evaluated following SOP# C1610 “Stability assessment of the cryopreserved LV-NY-ESO1 TCR sr39TK transduced autologous CD34+cells”.

10.3.1. The long-term stability of the frozen cell product was evaluated after storage in the vapor phase of LN2 (≤-140°C) for 30±7 days (1 month), 90±7 days (3 months), 180±10 days (6 months). The time point of 360±14 days (1 year) has not been evaluated as of the time of this report but will be evaluated in the future and the report will be amended to include this data. In the last two runs, (HD4 and HD5), the cell product thawed shortly (1-3 days) after cryopreservation (TCP day 0) was assessed as well. The number of time points evaluated from each cell product was dependent on the number of cells available. The Table 1 below summarizes the time points evaluated from each run. Cell product was thawed at 37°C and samples were taken to determine the quality attributes of the thawed cell product and to compare to those of the fresh cell product.

Table 1: Long term stability time points evaluated in donors HD1-HD5.

	FCP	TCP Day 0	TCP Day 30	TCP Day 90	TCP Day 180
HD1	✔		✔	✔	✔
HD2	✔		✔	✔	
HD3	✔		✔		✔
HD4	✔	✔	✔		
HD5		✔	✔		

10.3.2. To evaluate the post-thaw stability over a 48-hour period, the thawed cell product was split into two cryo-bags. One bag was stored at ambient room temperature and the other was refrigerated in a temperature-monitored refrigerator. At each of the 3, 6, 24 and 48-hour time points, 1.5-3 mL samples were taken from each of the bags and evaluated for TNC numbers, viability, CD34+ cell numbers and CFU potential.

10.4. Quality assessment tests

Quality parameters were asessed as follows:

10.4.1. Sterility was assessed by bacterial and fungal cultures for 14 and 21 days, respectively. The samples were submitted following SOP# 2100-Sample submission to clinical microbiology lab.

10.4.2. Endotoxin levels were assessed by using the Endosafe®-PTS^{TM} test system which utilizes the LAL kinetic chromogenic methodology. The system consists of a spectrophotometer and FDA-licenced Endosafe®-PTS^{TM} test cartridges that contain the precise amount of LAL reagent, chromogenic substrate and control standard endotoxin (CSE). The test system measures a color intensity that is directly related to the endotoxin concentration in a sample. The test was done following SOP# 2400 - Endotoxin Assay using Endosafe PTS.

10.4.3. Mycoplasma levels were assessed by using the MycoAlert™ Mycoplasma Detection Kit, a biochemical test that exploits the activity of mycoplasmal enzymes. The test was done following SOP# 2500- Mycoplasma Assay using MycoAlert™.

10.4.4. Total nucleated cell viability was asessed by trypan blue exlusion assay (SOP C1610 - LV-NY-ESO1 TCR sr39TK Autologous CD34+ stability asessment).

10.4.5. CD34+ cell recovery was calculated by dividing the "post-cryopreservation CD34+ cells/ml" by "fresh CD34 cells/ml" for long term stability study or by "0h CD34 cells/ml" for 3, 6, 24 and 48 h post-thaw time points and multiplying by 100%.

10.4.6. CD34+ cell enumeration was performed using the single-platform ISHAGE flow cytometric method following the testing facility SOP.

10.4.7. CFU potential was assessed by culturing CD34+ cells in a methylcellulose based medium for 13-15 days followed by colony enumeration and characterization based on their unique morphology. The assay was performed following SOP# C1800-Colony Forming Unit (CFU) Assay In Methylcellulose for human CD34+ cells.

10.4.8. Transduction efficiency

10.4.8.1. The vector copy number (VCN) of integrated lentivirus per cell of the bulk cell culture was assessed by plating 5×10^4 cells in media promoting myeloid differentiation and culturing for 14 days. The cells were harvested and genomic DNA was isolated (SOP# C1300-Total DNA extraction for VCN analysis) to determine average vector copy number (VCN) per cell by droplet digital PCR (ddPCR) (SOP# C1200-VCN quantification by ddPCR).The average VCN in the cell product was determined by normalizing the HIV-1 packaging signal sequence (Psi) to the cellular autosomal gene syndecan 4 (SDC4) as described in Cooper *et al.* J Viral Methods, 2011.

10.4.8.2. The Percentage of LV-NYESO positive CFUs was assessed following the CFU enumeration procedure. Single CFUs were individually picked for genomic DNA isolation (SOP# C1500-Genomic DNA extraction from Colony Forming Units) and determination of VCN/cell by ddPCR (SOP# C1200-VCN quantification by ddPCR).

10.4.8.3. Intracellular Vbeta 13.1 chain expression (TCR β chain variant specific for NY-ESO-1 TCR) was assessed by flow cytometry per SOP # C1900-Evaluation of the transgene TCRVbeta13.1 expression on transduced Stem Cells.

11. STATISTICAL ANALYSIS

Parameters regarding the manufacturing and *in vitro* characterization of the final cell products were tabulated and reported for each donor. Descriptive statistics such as mean and standard deviation were calculated. Linear mixed modeling approach was adopted to evaluate the long-term (Tables 6-8, Figure 1) stability and short-term stability (Figure 3) of the cryopreserved cell products. Specifically, donor-to-donor variations were modeled through random intercepts, while time was treated as a fixed effect. Within the linear mixed model framework, an overall test of time effect was performed, followed by Tukey-Kramer adjusted pairwise comparisons. All statistical tests for significance were two-tailed. A p-value less than the 0.05 significance level was considered to be statistically significant. All statistical analyses were carried out using SAS version 9.4 (SAS Institute Inc. 2013).

12. RESULTS

12.1. Long-term stability of the cryopreserved cell product after 30, 90 and 180 days post cryopreservation.

To determine the stability of the cell product during cryopreservation, five independent LV-NYESO TCR/sr39TK PBSC cell products were manufactured and underwent stability assessment. The time points at which stability was assessed were based on cell availability.

The results for the recovery of cell product of the individual donors are summarized in Tables 1-5. The parameters that didn't meet the acceptance criteria as defined in protocol AR-CIRM-17-002-P are indicated in each of the tables.

Table 6 summarizes the parameters that reflect cell product stability i.e. viability, CD34 recovery and clonogenic potential before and after 30, 90 and 180 days following

cryopreservation of the cell product. The data indicates that the cryopreservation didn't affect any of these parameters at the time points tested.

The cryopreservation also did not affect the distribution of erythroid (BFU-E/CFU-E), myeloid (CFU-G/M/GM) and multi-lineage (CFU-GEMM) colonies (p values are 0.35, 0.66 and 0.46 respectively)(Figure 1). The transduction level of the recovered cell product was not significantly changed during the storage in vapor phase LN, as measured by Vb13.1, average VCN and % of LV-NYESO TCR/sr39TK positive CFUs (Table 7 and Figure 2). In addition no significant difference was observed in parameters defining cell product purity i.e. endotoxin, mycoplasma sterility and percentage of CD34+ cells (Table 8).

Table 1: HD1-Comparison between fresh and cryopreserved product at 30, 90 and 180 days.

Test	Method	Specifications	FCP	TCP 30d	TCP 90d	TCP 180d
CD34+ cell enumeration (cells/ml)	Flow Cytometry (ISHAGE)	Record results	3.0×10^6	2.7×10^6	2.8×10^6	2.5×10^6
% CD34 cells (purity)	Flow Cytometry (ISHAGE)	≥50%	98.66	97.46	98.93	97.16
%TNC viability	Trypan Blue manual count	≥70%	99.5	98.5	98.1	98.0
% CD34+ cell recovery		Record results	100	90.0	93.3	83.3
Endotoxin	Endosafe®PTS	≤ 5 EU/Kg	0.095	0.058	0.072	0.058
Mycoplasma	MycoAlert test	Ratio<0.9	0.18	0.50	0.53	0.22
Sterility	Sterility culture	No growth	No growth	No growth	No growth	No growth
	Fungal culture	No growth	No growth	No growth	No growth	No growth
%Vβ13.1 positive cells	Flow Cytometry	Record results	18.8	16	20.3	9.85
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	51±14	58±2	53±13	51±11
% LV-NYESO TCR/sr39TK positive CFU	ddPCR	Record results	35.4	44	24.5	26
Vector copy number (VCN)	ddPCR and/or qPCR	Record results	0.1	0.1	0.3	0.1

Table 2: HD2-Comparison between fresh and cryopreserved product at 30 and 90 days.

Test	Method	Specifications	FCP	TCP 30d	TCP 90d
CD34+ cell enumeration (cells/ml)	Flow Cytometry (ISHAGE)	Record results	1.4×10^6	1.1×10^6	1.2×10^6
% CD34 cells (purity)	Flow Cytometry (ISHAGE)	≥50%	93.14	96.78	94.09
%TNC viability	Trypan Blue manual count	≥70%	97.2	98.2	96.0
% CD34 recovery		Record results	100	78.6	85.7
Endotoxin	Endosafe®PTS	≤ 5 EU/Kg	0.132	0.132	0.132
Mycoplasma	MycoAlert test	Ratio<0.9	0.4	0.2	0.2
Sterility	Sterility culture	No growth	No growth	No growth	No growth
	Fungal culture	No growth	No growth	No growth	No growth
%Vβ13.1 positive cells	Flow Cytometry	Record results	13.2%	3.7%#	18.6
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	64±4	44±6	42±5
% LV-NYESO TCR/sr39TK positive CFU	ddPCR	Record results	21.4	29.6	16.5
Vector copy number (VCN)	ddPCR and/or qPCR	Record results	0.1	0.3	0.1

Vb13.1 expression level for this time point iis lower than the other time points for this cell product. This value was incorporated in the average Vb13.1 percentage calculations (see table 7).

Table 3: HD3-Comparison between fresh and cryopreserved product at 30 and 180 days.

Test	Method	Specifications	FCP	TCP 30d	TCP 180d
CD34+ cell enumeration (cells/ml)	Flow Cytometry (ISHAGE Protocol)	Record results	2.5×10^6	2.1×10^6	2.8×10^6
% CD34 cells (purity)	Flow Cytometry (ISHAGE Protocol)	≥50%	97.68	88.75	97.84
%TNC viability	Trypan Blue manual count	≥70%	98.6	98.9	97.4
% CD34 recovery		Record results	100	84.0	112.0
Endotoxin	Endosafe®PTS	≤ 5 EU/Kg	0.139	0.139	0.139
Mycoplasma	MycoAlert test	Ratio<0.9	0.46	0.31	0.34
Sterility	Sterility culture	No growth	No growth	No growth	No growth
	Fungal culture	No growth	No growth	No growth	No growth
%Vβ13.1 positive cells	Flow Cytometry	Record results	24.9%	18%	9.18%
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	52±10	47±11	46±3
% LV-NYESO TCR/sr39TK positive CFU	ddPCR	Record results	28.7	19	25.8
Vector copy number (VCN)	ddPCR and/or qPCR	Record results	0.3	0.3	0.3

Table 4: HD4-Comparison between fresh and cryopreserved product at 0 and 30 days.

Test	Method	Specifications	FCP	TCP 0d	TCP 30d
CD34+ cell enumeration (cells/ml)	Flow Cytometry (ISHAGE)	Record results	1.5×10^6	1.1×10^6	1.5×10^6
% CD34 cells (purity)	Flow Cytometry (ISHAGE)	≥50%	94.14	95.5	97.04
%TNC viability	Trypan Blue manual count	≥70%	88.8	91.0	86
% CD34 recovery		Record results	100	73%	100%
Endotoxin	Endosafe®PTS	≤ 5 EU/Kg	0.129	0.181	0.097
Mycoplasma	MycoAlert test	Ratio<0.9	0.23	0.21	0.23
Sterility	Sterility culture	No growth	No growth	No growth	No growth
	Fungal culture	No growth	No growth	No growth	No growth
%Vβ13.1 positive cells	Flow Cytometry	Record results	30.9	32.5	37.2
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	36±1	33±6	36±6
% LV-NYESO TCR/sr39TK positive CFU	ddPCR	Record results	31.4	43	30
Vector copy number (VCN)	ddPCR and/or qPCR	Record results	0.4	0.4	0.4

Table 5: HD5-Comparison between cryopreserved cell product at day 0 and 30 days.

Test	Method	Specifications	TCP 0d	TCP 30d
CD34+ cell enumeration (cells/ml)	Flow Cytometry (ISHAGE)	Record results	1.4×10^6	1.5×10^6
% CD34 cells (purity)	Flow Cytometry (ISHAGE)	≥50%	96.27	97.95
%TNC viability	Trypan Blue manual count	≥70%	96.2	95.2
% CD34 recovery[#]		Record results	93.3%	100%
Endotoxin[#]	Endosafe®PTS	≤ 5 EU/Kg	0.101	0.155
Mycoplasma	MycoAlert test	Ratio<0.9	0.07	0.39
Sterility	Sterility culture	No growth	No growth	No growth
	Fungal culture	No growth	No growth	No growth
%Vβ13.1 positive cells	Flow Cytometry	Record results	31.4%	35.3%
CFU potential	Methylcellulose culture (%CFU/cells plated)	Record results	46±10	39±4
% LV-NYESO TCR/sr39TK positive CFU	ddPCR	Record results	37%	36%
Vector copy number (VCN)*	ddPCR and/or qPCR	Record results	0.4	0.5

[#] For this run %CD34 recovery is calculated based on cell concentration of CD34+ cells prior to cryopreservation (1.5×10^6 cells/ml).

Table 6: Long term stability parameters

Tests	FCP N=4	TCP 0 days N=2	TCP 30days N=5	TCP 90 days N=2	TCP 180 Days N=2	P value
TNC viability (%)	96.03±4.9	93.6±3.4	95.4±5.4	97.1±1.5	97.7±0.4	0.17
CD34 recovery (%)	100	83.15±14.35	90.52±9.55	89.50±5.37	97.65±20.29	0.31
CFU potential (%)	51±11	40±9	45±9	48±8	49±4	0.67

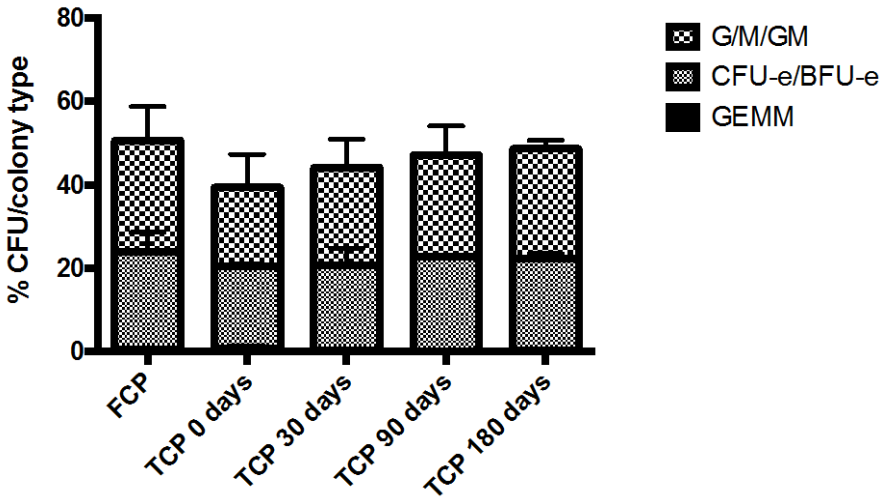


Figure 1. The percentages of the different types of hematopoietic colonies identified are presented. Data is presented as mean±SD and grouped by sample type.

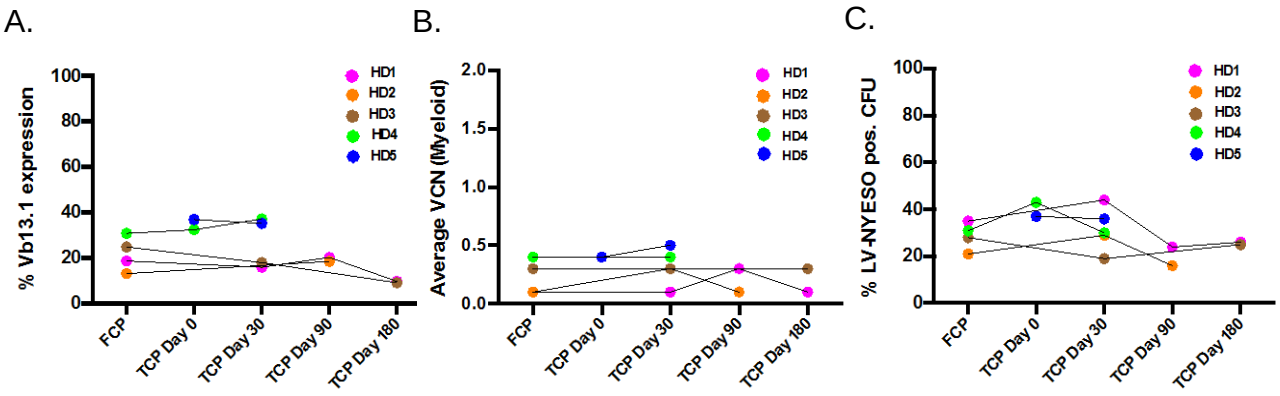


Figure 2. Transduction stability summary: (A) Vbeta13.1, NYESO TCR β chain variant expression was assessed by intracellular staining followed by flow cytometry analysis. (B) Average VCN of LV-NYESO vector in CD34+ cells after 14 days in culture in media promoting myeloid differentiation was assessed by ddPCR. (C) Percentage of LV-NYESO positive CFU assessed at different time points post cryopreservation by single colony ddPCR. Each of the cell products is color coded as represented in the key.

Table 7: Transduction stability summary

Test	FCP N=4	TCP 0 days N=2	TCP 30days N=5	TCP 90 days N=2	TCP 180 Days N=2	P value
VCN	0.2±0.2	0.4±0.0	0.3±0.1	0.2±0.1	0.2±0.1	0.80
Vb13.1 (%)	21.95±7.64	31.95±0.78	26.63±1.2	19.45±1.2	9.52±0.47	0.15
LV-NYESO pos. CFU (%)	29.2±5.9	40.0±4.2	31.7±9.2	20.5±5.7	25.9±0.1	0.15

The results in the table are mean ± SD.
FCP-Fresh cell product; TCP-thawed cell product.

Table 8: Purity parameter summary

Test	FCP N=4	TCP 0 days N=2	TCP 30days N=5	TCP 90 days N=2	TCP 180 Days N=2	P value
% CD34+ cells (purity)	95.91±2.68	95.89±0.54	95.60±3.85	96.51±3.42	97.50±0.48	0.95
Endotoxin (EU/kg)	0.12±0.02	0.14±0.06	0.12±0.04	0.10±0.04	0.10±0.06	0.88
Mycoplasma	0.32±0.13	0.14±0.10	0.33±0.12	0.37±0.23	0.28±0.08	0.53
Sterility culture (14 days)	Neg	Neg	Neg	Neg	Neg	N/A
Fungal culture (21 days)	Neg	Neg	Neg	Neg	Neg	N/A

The numerical results in the table are mean ± SD.

FCP-Fresh cell product; TCP-thawed cell product.

12.2. Short-term stability of the cryopreserved cell product after 3, 6, 24 and 48 hours post thaw. The original data is summarized in tables 7-11. The discrepancies in the procedures are indicated in each of the tables and summarized in section 13.

Table 9: HD1 Post-thaw stability assessment

A. After 30 days post-cryopreservation

Test	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	98.5	97.0	96.9	95.2	97.5	92.4	97.3	82.1	92.9
CD34+ cell viability (%)	93.82	87.13	92.01	89.29	86.58	84.16	91.39	60.88	84.99
CD34+ cells/ml	2.7×10^6	1.8×10^6	3.6×10^6	2.1×10^6	2.2×10^6	1.7×10^6	1.7×10^6	1.4×10^6	1.7×10^6
CD34+ cell recovery (%)	N/A	67	133	78	81	63	63	52	67
CFU potential (%)	58±2	50±9	47±6	41±8	43±10	14±1	43±5	5±1	30±6

B. After 90 days post-cryopreservation

Test	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	98.1	98.2	98.2	97.5	97.5	96.1	98.6	89.0	96.8
CD34+ cell viability (%)	94.35	92.76	92.35	91.52	88.97	86.18	89.49	78.07	89.24
CD34+ cells/ml	2.8×10^6	2.6×10^6	2.8×10^6	2.6×10^6	2.6×10^6	2.9×10^6	2.5×10^6	1.2×10^6	2.0×10^6
CD34+ cell recovery (%)	**N/A**	93	100	93	93	104	89	43	71
CFU potential (%)	53±13	48±1	55±10	47±8	46±3	16±1	23*	10±3	28±3

* No duplicate plate was counted. #Discrepancy filed: DIS-01 (see section 13 for details)

C. After 180 days post-cryopreservation

Test	0h	3h		6h		24h		48h*	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	98.0	97.3	97.8	97.9	97.9	92.7	97.6	88.1	95.7
CD34+ cell viability (%)	89.49	90.31	87.13	87.85	87.53	78.57	87.91	**N/A**	**N/A**
CD34+ cells/ml	2.5×10^6	2.4×10^6	2.6×10^6	2.1×10^6	2.1×10^6	2.3×10^6	2.4×10^6	**N/A**	**N/A**
CD34+ cell recovery (%)	**N/A**	96	104	84	84	92	96	**N/A**	**N/A**
CFU potential (%)	51±11	52±7	53±9	38±3	50±14	35±4	43±6	9±1	26±2

*CD34 enumeration was not performed after 48h post-thaw (180 days post-cryopreservation).

Discrepancy filed: DIS-02 (see section 13 for details)

Table 10: HD2 Post-thaw stability assessment

A. After 30 days post-cryopreservation

Tests	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	98.2	95.2	98.0	96.4	98.9	87.05	92.65	62.4	91.6
CD34+ cell viability (%)	89.10	85.37	83.25	84.83	82.76	55.92	73.83	15.43	65.97
CD34+ cells/ml	1.1×10^6	1.2×10^6	1.1×10^6	1.0×10^6	1.0×10^6	0.8×10^6	1.0×10^6	0.2×10^6	0.9×10^6
CD34+ cell recovery (%)	N/A	109	100	91	91	73	91	18	82
CFU potential (%)	44±6	37±7	44±5	49±3	42±6	3±1	17±2	0±0	9±3

B. After 90 days post-cryopreservation

Tests	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	96.0	95.5	96.6	93.4	96.5	92.0	96.2	73.3	94.5
CD34+ cell viability (%)*	88.67	83.7	82.5	87.5	86.1	74.3	78.5	35.2	70.4
CD34+ cells/ml*	1.2×10^6	1.0×10^6	1.1×10^6	2.4×10^6	2.4×10^6	1.6×10^6	2.2×10^6	0.4×10^6	1.8×10^6
CD34+ cell recovery (%)*	N/A	85	85	185	185	123	169	31	138
CFU potential (%)	42±5	31±6	36±5	37±7	42±6	8±2	18±0	0.3±0	9±2

***** CD34 enumeration was done in a different facility and was not included in the final summary (Table 13, Figure 3). Discrepancy filed: DIS-03 (see section 13 for details)

Table 11: HD3 Post-thaw stability assessment

A. After 30 days post-cryopreservation

Tests	0h	3h		6h		24h		48h	
		RT	4°C	RT	RT	RT	RT	4°C	RT
TNC viability (%)	98.9	96.6	96.7	95.8	97.6	92.9	96.7	76.3	95.3
CD34+ cell viability (%)*	89.02	84.0	79.5	86.5	88.2	69.1	77.7	32.8	55.4
CD34+ cells/ml*	2.1×10^6	1.7×10^6	1.9×10^6	2.0×10^6	2.1×10^6	1.4×10^6	1.7×10^6	0.6×10^6	1.2×10^6
CD34+ cell recovery (%)*	N/A	81	90	95	100	67	81	29	57
CFU potential (%)	47±11	45±3	46±3	43±8	49±10	14±1	27±4	1±1	15±15

***** CD34 enumeration was done in a different facility and was not included in the final summary (Table 14, Figure 3). Discrepancy filed: DIS-03 (see section 13 for details)

B. After 180 days post-cryopreservation

Tests	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	97.4	98.2	98.1	98.3	98.0	96.3	97.2	88.8	95.9
CD34+ cell viability (%)	94.05	90.41	90.80	91.32	90.27	82.96	88.57	53.43	81.25
CD34+ cells/ml	2.8×10^6	2.2×10^6	2.2×10^6	2.3×10^6	2.4×10^6	2.1×10^6	2.6×10^6	1.4×10^6	2.3×10^6
CD34+ cell recovery (%)	N/A	79	79	82	86	75	93	50	82
CFU potential (%)	46±3	42±4	42±5	35±4	48±6	13±3	34±4	1±0.5	17±5

Table 12: HD4 Post-thaw stability assessment

After 30 days post-cryopreservation

Tests	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	86.0	86.7	89.4	87.4	90.3	87.1	88.4	77.7	86.1
CD34 viability (%)	90.73%	88.61	85.67	79.53	80.78	70.62	81.85	26.98	75.43
CD34+ cells/ml	1.5×10^6	1.4×10^6	1.4×10^6	1.6×10^6	1.4×10^6	1.3×10^6	1.4×10^6	0.4×10^6	1.2×10^6
CD34+ cell recovery (%)	**N/A**	93	93	107	93	87	93	27	80
CFU potential (%)	36±6	33±6	35±4	22±3	26±3	10±1	21±4	0	14±2

Table 13: HD5 Post-thaw stability assessment

After 30 days post-cryopreservation

Tests	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC viability (%)	95.2	93.2	95.05	92.1	94.5	90.3	94.7	83.0	94.4
CD34 viability (%)	85.12	89.38	87.08	87.04	84.33	70.36	80.14	24.60	75.76
CD34+ cells/ml	1.5×10^6	1.6×10^6	1.5×10^6	1.5×10^6	1.3×10^6	1.1×10^6	1.3×10^6	0.4×10^6	1.3×10^6
CD34+ cell recovery (%)	N/A	107	100	100	87	73	87	27	87
CFU potential (%)	39±4	40±5	42±6	40±6	43±7	6±0	26±5	0±0	12±2

The results of the post-thaw stability are summarized in Figure 3 and table 14. Based on the results of TNC and CD34 viability, percentage of CD34 recovery and percentage of CFU/cells plated, the cell product is stable for up to 6 hours. The clonogenic potential of the thawed cell

product and CD34+ cell viability are significantly decreased after 24h storage in both storage conditions: ambient temperature (RT) and refrigeration (Figure 3 B and D).

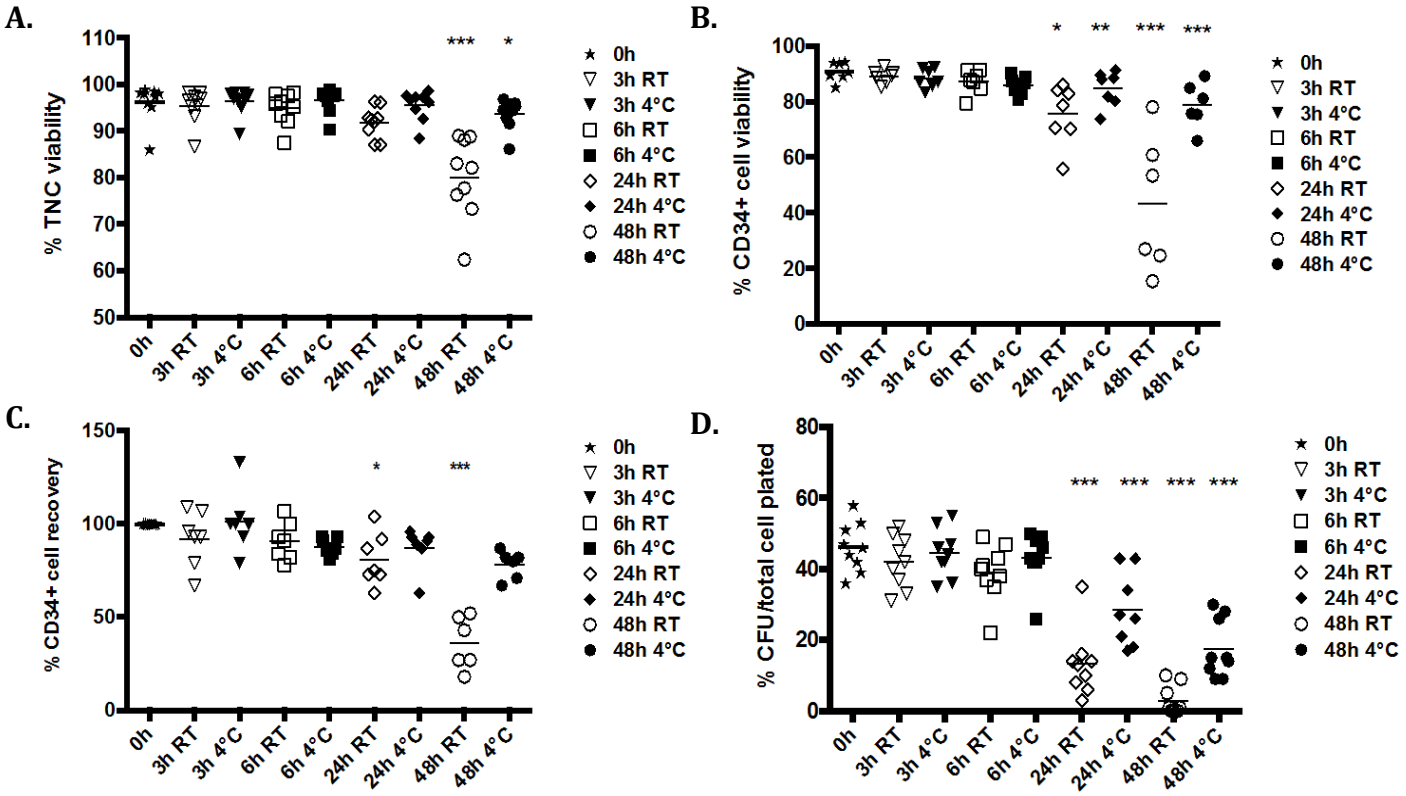


Figure 3: Post-thaw stability of the cell product. The graphs summarize cumulative data from five independently manufactured cell products. The stability parameters of the cell product stored at RT or at 4°C were analyzed after 3, 6, 24 and 48h post-thaw and compared to those at 0h. (A) Total nucleated cell (TNC) viability of the TCP at different time points after thawing. (B) CD34+ cell viability of the FCP at different time points after thawing (N=9). (C) CD34+ cell recovery of the FCP at different time points after thawing (N=7). (C) CD34+ cell recovery was calculated by dividing the "number of CD34+ cells/ml at 0h" by the "number of cells/ml at 3, 6, 24 and 48h time points" and multiplying by 100% (N=7). (D) Clonogeneic potential of the TCP after thawing, as measured by %CFU per number of plated cells (N=9). Horizontal bars show averaged values (*p<0.05, **p<0.01 and ***p<0.001 when compared to 0h time point, by Tukey-Kramer test)

Table 14: Summary of the post-thaw cell product stability parameters

Test	0h	3h		6h		24h		48h	
		RT	4°C	RT	4°C	RT	4°C	RT	4°C
TNC Viability (%)	96±4	95±4	96±3	95±3	97±3	92±3	95±3	80±9	94±3
CD34+ cell viability (%)	91±3	89±2	88±3	87±4	86±3	76±11	85±6	43±25	79±8
[#]CD34+ cell recovery (%)	100±0	92±15	101±16	91±10	88±5	81±14	87±11	36±14	78±8
% CFU/total cell plated	46±7	42±7	44±7	39±8	43±7	13±9	29±10	3±4	18±8

[#]The percentage of CD34+ cell recovery was calculated by dividing the "number of CD34+ cells/ml at 0h" by the "number of cells/ml at 3, 6, 24 and 48h time points" and multiplying by 100%. All the data points are compared to the data at 0h. Results are mean ± SD

13. DISCREPANCIES

A total of three protocol discrepancies occurred during the execution of this qualification study. The associated completed DIS forms are included as Attachments 15.1.1-15.1.3

***DIS-01*:** The CFU enumeration assay for HD1, 90 days, 24h post-thaw at 4°C was not counted in duplicate due to dried up methylcellulose in one of the plates. As a result, no SD was calculated for this time point and this value was omitted from the overall summary (see figure 3 D). Since 8 other CFU samples were evaluated at the same time point (for HD2-HD5) and under the same conditions, this discrepancy was determined to have no impact on the overall short-term stability assessment of the cell product (Attachment 15.1.1).

***DIS-02*:** The CD34+ cell enumeration assay for HD1, 180 days post-cryopreservation, 48h post-thaw was not performed. The testing core facility performing the CD34+ cell enumeration assay was changed from the UCLA Bone Marrow Transplant Unit Flow Cytometry Lab to the UCLA

Immunogenetics Center Immune Assessment Core. The new core facility that was used for CD34+ enumeration needed additional samples to validate the assay, therefore, there was not enough sample left to perform the CD34+ cell enumeration at the 48h time-point (post thaw). Since 6 other samples were assessed for CD34+ cell enumeration at the same time point and under the same conditions, this discrepancy was determined to have no impact on the overall short-term stability assessment of the cell product (Attachment 15.1.2).

***DIS-03*:** The testing core facility performing the CD34+ cell enumeration assay was changed from the UCLA Bone Marrow Transplant Unit Flow Cytometry Lab to the UCLA Immunogenetics Center Immune Assessment Core. During the transition period, the samples for HD2, 90 days post-cryopreservation and HD3 30 days post-cryopreservation at 3, 6, 24 and 48h post-thaw were performed "in house". Due to differences in the analysis methodology, the CD34 enumeration results from those time points were not included in the final summary (see Figure 3B and 3C). Since 7 other samples were assessed for CD34+ cell enumeration at the same time points (6 samples for the 48h time point) and under the same conditions, this discrepancy was determined to have no impact on the overall short term stability assessment of the cell product (Attachment 15.1.3).

14. CONCLUSIONS

The current report summarizes the results of the long and short-term stability studies for cryopreserved LV-NYESO TCR/sr39TK PBSC product.

These results show that the cell product is stable for at least 180 days after cryopreservation and storage in a vapor phase of liquid nitrogen. The short-term stability results show that the cell product is stable up to 6 hours post-thaw.

15. ATTACHMENTS

15.1. Discrepancy Evaluation Forms:

15.1.1. DIS-01

15.1.2. DIS-02

15.1.3. DIS-03

15.2. Discrepancy log

15.3. AR-CIRM-17-002-P ver.01: Stability evaluation of PBSC transduced with LV-optNYESO TCR/sr39TK.