Supplemental Materials

NY-ESO-1 specific TCR engineered T-cells mediate sustained antigen-specific antitumor effects in myeloma

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Supplementary Table 1: Patient demographics and status on the study. 70% of the patients were Caucasian, 30% were African-American or Hispanic. Fifty percent of the patients were female and the median age at enrollment was 59 (range: 46-72). Patients received a median of 3 lines of prior therapy (range: 1-5); 5 patients (25%) had relapsed after an earlier ASCT. Patients with high risk disease were typically enrolled after receiving their initial line of therapy. Twelve patients (60%) had cytogenetic abnormalities including 7 (35%) with confirmed high-risk abnormalities [t(4;14), del17p13,complex]. Patient responses are shown: Best response prior to infusion ("Pre Inf Resp" column), and responses at day 42, 100 and 180 post infusion, are shown in the last four columns. Administration of protocol-mandated lenalidomide starting at day 100, is noted ("Len. @ dy 100 (Y/N)" column).

Study ID	Sex	Race	Age at Screen	Year Dx	# prior Rx	Prior ASCT	Prior duration of response post ASCT	Chrom Abs	Date of Infusion	Len. @ dy 100 (Y/N)	Pre Inf Resp	Day 42 Resp	Day 100 Resp	Day 180 Resp
01411-250	M	Cauc	72	2006	4	Y	2yr 8mo	t4:;14	30-Jun-11	Y	PD	sCR	sCR	sCR
01411-200	M	Cauc	61	2011	1	N	NA	17p del, trisomy 11, p53 overexpr ession	26-Aug-11	Y	PD	nCR	nCR	nCR
01411-201	F	Cauc	55	2011	1	N	NA	t4;14	31-Oct-11	Y	PR	nCR	nCR	nCR
01411-252	M	Cauc	72	2002	4	Y	4 yr	loss chr 13, del14, del17	5-Jan-12	N	PR	PR	PR	PR
01411-253	F	Black	46	2010	2	Y	7.5 mos	complex	19-Jan-12	Y	PD	nCR	nCR	PD
01411-204	F	Hisp / Cauc	66	2010	4	N	NA	none	3-Feb-12	Y	PR	VGPR	nCR	PD
01411-206	M	Cauc	63	2011	1	N	NA	14 transloca tion	23-Mar-12	Y	VGPR	VGPR	VGPR	VGPR
01411-254	F	Cauc	49	2010	4	N	NA	monoso my 13, trisomy 11 at diagnosis . None at time of screen.	29-Mar-12	Y	SD	PR	PR	PD

Study ID	Sex	Race	Age at Screen	Year Dx	# prior Rx	Prior ASCT	Prior duration of response post ASCT	Chrom Abs	Date of Infusion	Len. @ dy 100 (Y/N)	Pre Inf Resp	Day 42 Resp	Day 100 Resp	Day 180 Resp
01411-207	F	Black	66	2011	1	N	NA	13 del	13-Apr-12	Y	PR	VGPR	nCR	nCR
01411-202	F	Black	47	2011	1	N	NA	13 del	20-Apr-12	N	PR	PR	nCR	nCR
01411-255	M	Cauc	59	2011	2	N	NA	none	11-May-12	N	SD	PR	nCR	nCR
01411-208	M	Cauc	44	2006	5	Y	4 yr 4 mo	none	NA	NA	NA	NA	NA	NA
01411-209	M	Cauc	64	2011	3	N	NA	none	13-Jul-12	N	SD	VGPR	nCR	nCR
01411-251	F	Cauc	57	NA	NA	NA	NA	UNK	NA	NA	NA	NA	NA	NA
01411-203	M	Cauc	56	2011	1	N	NA	none	NA	NA	NA	NA	NA	NA
01411-205	F	Cauc	50	2009	7	Y	10 mo	none	NA	NA	NA	NA	NA	NA
01411-210	M	Black	53	2011	2	N	NA	none	17-Aug-12	Y	SD	VGPR	VGPR	VGPR
01411-211	F	Cauc	66	2012	3	N	NA	UNK	24-Aug-12	Y	SD	VGPR	nCR	nCR
01411-256	M	Cauc	54	2011	2	N	NA	none	30-Aug-12	Y	nCR	VGPR	nCR	CR
01411-257	M	Cauc	59	2012	2	N	NA	NA	4-Dec-12	N	VGPR	SD	PD	NA
01411-258	M	Cauc	47	2010	4	Y	15 mon	complex karyotype with loss of 6q, gain of 1q21, loss of 13q14 by G banding & FISH analysis	6-Jan-13	Y	PD	nCR	nCR	nCR
01411-212	M	Cauc	70	2012	1	N	NA	deletion-Y in 8 of 20 cells. positive for deletion 13q14	15-Feb-13	N	PR	nCR	CR	CR
01411-259	M	Cauc	50	2012	4	Y	6 mo	Deletion 17p13 in 5 out of 23 cells. Deletion 13q14, trisomy 3, and perhaps a t4;14 translocation.	18-Apr-13	Y	PD	nCR	sCR	sCR
01411-260	F	Black	59	2011	*1*	N	NA	none	1-Aug-13	N	SD	SD	SD	SD

Supplementary Table 2. Phenotypic analysis of cell product. The NY-ESO^{c259} TCR contains a Vβ13.1 chain, which is used to detect CD4 and CD8 T cells that carry the TCR. Approximately 1-5% of non-gene-modified cells will also carry the Vβ13.1 chain and therefore transduction rates are slightly lower than shown. Patient 260 product was analyzed using a different flow cytometry panel, and therefore the percentage of CD4 and CD8 is shown in the Vβ13.1 positive population and these numbers are excluded from the summary analysis.

Patient ID	CD3%	%CD62L in CD3	%CD3+Vβ13.1	%CD4 in CD3	%CD8 in CD3
250	98.5	62.6	46.9	NA	NA
200	87.3	95.2	34.3	76.4	31.9
201	99.5	98.6	34.3	54.8	47.6
252	95.9	89.4	30.0	34.8	67.3
253	98.5	98.3	23.5	26.3	75.2
204	98.3	98.0	26.6	80.5	21.2
206	99.3	98.7	34.6	52.0	65.9
254	99.9	93.5	33.2	52.1	47.2
207	99.6	NA	37.8	83.7	24.9
202	99.8	96.2	37.4	37.8	62.6
255	99.9	88.1	31.4	74.5	24.6
209	99.8	87.6	18.2	80.1	24.0
210	99.4	92.0	33.8	66.1	43.7
211	99.5	98.1	29.9	66.9	34.6
256	99.8	85.5	41.9	51.5	59.0
257	99.8	NA	31.0	60.0	41.0
258	99.7	97.0	27.0	82.0	18.0
212	99.3	45.4	37.6	69.0	30.9
259	99.8	86.9	49.1	49.8	51.1
260	98.6	ND	29.3	47.5	20.3
Median	**99.5**	**93.5**	**33.5**	**63.1**	**42.4**
Minimum	**87.3**	**45.4**	**18.2**	**26.3**	**18.0**
Maximum	**99.9**	**98.7**	**49.1**	**83.7**	**75.2**

%CD4 in Vβ 13.1

%CD8 in Vβ 13.1

Supplementary Table 3. Evaluation of gene modified cell persistence in blood by PCR during long term follow-up. 7/10 have detectable cells above the limit of detection, but below the limit of quantitation (LOQ) for the assay. Two patients have quantifiable levels (<1% and 2%).

	Vector copies per microgram DNA (percent marking)		
Patient ID	**Year 2**	**Year 2.5**	**Year 3**
201	*missing*	3800.8 (2.39)	4511.7 (2.84)
252	465.0 (0.29)	531.5 (0.33)	
206	Positive but <LOQ	Positive but <LOQ	
254	Negative	Negative	
207	Positive but <LOQ	Positive but <LOQ	
255	Positive but <LOQ		
209	Positive but <LOQ		
210	Positive but <LOQ		
211	Positive but <LOQ		
256	Positive but <LOQ		

Supplementary Table 4. Elevation of IL-6 in patients following T cell infusion. The cytokines ILI-β, IL-10, IL-13, IL-6, IL-12, IL-17, GM-CSF, IL-15, IL-5, IFN-γ, IFN-α were routinely monitored in patients. Of these, IL-6 was increased in nearly all patients, to levels that are higher than expected with transplantation alone (5—10 pg/ml)[1], and are comparable to levels observed with CD19-41BB CAR [2,3], despite the absence of clear clinical signals for CRS in our study.

Patient ID	pre	day 3 or 5	day 7	day 14	day 21	day 28	peak day	max fold increase
250	7.27	5.5	21.05	257.77	13880.65	ND	21	1909
200	6.25	11.58	88.71	139.17	26.06	7.70	14	22.3
201	4.39	9.36	146.13	26.79	22.02	39.23	7	33.3
252	2.48	17.24	74.94	ND	16.69	8.07	7	30.2
253	2.13	4.74	4839.30	45.03	8.06	2.23	7	2272
204	8.65	9.34	2208.51	20.61	17.68	20.34	7	255
206	4.13	7.97	45.49	3.94	4.14	4.80	7	11.0
254	3.40	2.17	11.11	37.02	2.45	2.50	14	10.9
207	2.15	5.40	35.27	4.86	10.38	215.02	28	100
202	4.70	10.32	49.36	18.94	16.04	2.91	7	10.5
255	2.69	3.25	9.16	5.62	13.90	9.85	21	5.2
209	2.04	2.52	46.09	7.09	4.12	4.84	7	22.6
210	3.29	6.38	10.36	11.79	27.24	8.61	21	8.3
211	2.50	4.86	46.08	22.80	5.05	3.58	7	18.43
256	1.76	45.05	66.60	29.70	6.62	8.59	7	37.84
257	M	M	M	M	M	M	NA	NA
258	M	M	M	M	M	M	NA	NA
212	0.69	5.65	86.15	13.54	ND	ND	NA	NA
259	333.39	23.21	81.40	12.22	677.32	568.83	21	2.03
260	M	M	M	M	M	M	NA	NA
						Median	7	22

1. Condomines, M., et al. Increased plasma-immune cytokines throughout the high-dose melphalan-induced lymphodepletion in patients with multiple myeloma: a window for adoptive immunotherapy. Journal of immunology 184, 1079-1084 (2010).

2. Kalos, M., et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Science translational medicine 3, 95ra73 (2011).

3. Maude, S.L., et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. The New England journal of medicine 371, 1507-1517 (2014).

Supplementary Table 5. List of serious adverse events on the study.

Pt #	T-cell infusion date	Study dose	TD %	Date of onset	End date	Realday	Diagnosis	Grade	Outcome	Relationship
01411-202	20-Apr-12	7.87 billion	37.4%	23-Jul-12	30-Jul-12	94	Hypoxia	3	Recovered	Possible
01411-201	31-Oct-11	9.5 billion	34.0%	2-May-12	18-May-12	184	Neutropenia	4	Recovered	Possible
01411-204	3-Feb-12	8.82 billion	26.6%	21-Mar-12	26-Mar-12	47	Hyponatremia	4	Recovered	Possible
01411-209	13-Jul-12	9.46 billion	18.2%	19-Jul-12	27-Jul-12	6	Hypotension	3	Recovered	Possible
01411-209	13-Jul-12	9.46 billion	18.2%	9-Aug-12	19-Nov-12	27	Graft Versus Host Disease - GI	3	Recovered	Possible
01411-209	13-Jul-12	9.46 billion	18.2%	15-Oct-12	19-Nov-12	94	Pancytopenia	4	Recovered	Possible
01411-253	19-Jan-12	5.99 billion	23.5%	2-Feb-12	8-Feb-12	14	Dehydration	3	Recovered	Possible

Supplementary Table 6. List of adverse events at least probably related to the study.

Data cut off: 31st July 2013					
Number of events	**17**				
System Organ Class					
Preferred Term					
	Event (%)	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Allergy/Immunology	**5 (29)**				
Graft Versus Host Disease - GI	3 (60)	0 (0)	0	3 (100)	0 (0)
Graft Versus Host Disease-skin	2 (40)	0 (0)	2 (100)	0 (0)	0 (0)
Constitutional Symptons	**3 (18)**				
Fatigue	1 (33)	1 (100)	0	0 (0)	0 (0)
Fever	2 (67)	1 (50)	1 (50)	0 (0)	0 (0)
Skin and subcutaneous tissue disorders	**3 (18)**				
Rash	3 (100)	1 (33)	1 (33)	1 (33)	0 (0)
Gastrointestinal	**2 (11)**				
Diarrhea	2 (100)	1 (50)	1 (50)	0 (0)	0 (0)
Cardiac disorders	**1 (6)**				
Sinus tachycardia	1 (100)	1 (100)	0	0 (0)	0 (0)
Dermatology/Skin	**1 (6)**				
Injection site reaction/extravasation changes	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
General disorders and administration site conditions	**1 (6)**				
Weakness	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
Vascular disorders	**1 (6)**				
Hypotension	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)

Supplementary Table 7. Evaluation of gene marked cells in affected and non-affected colon biopsy samples.

	Specimen	% Vector$^+$ Cells/ Total Cells
Penn Subject #2	Abnormal Colon	0.12
	Normal Colon	2.9
	Skin Biopsy	24.8
	Peripheral Blood	4.5
Penn Subject #3	Rectosigmoid Colon	4.3
	Terminal Ileum	2.1
	Peripheral Blood	1.6
Penn Subject #8	Colon Biopsy	2
	Upper GI Biopsy	4.2

Supplementary Table 8. Patterns of relapse.

Patient ID	Best Response	Timepoint at relapse	Persistence - gene modified cells	NY-ESO Antigen	LAGE Antigen	Conclusions
250	sCR	1 year	68 cells/ul	0	0	cells present, antigen negative tumor on relapse
200	nCR	1 year	1 cells/uL	ND*	ND*	loss of persistence, likely antigen positive tumor based on surrounding timepoints
204	nCR	9 months	<5 cells/uL**	0.011	6.2431	loss of persistence, antigen positive tumor
253	nCR	3 months	3 cells /uL blood	0.02	67	loss of persistence, antigen positive tumor
254	PR	6 months	6 cells / uL blood	0	0	loss of cells, antigen negative tumor on relapse
202	nCR	1.6 year	<5 cells/uL	ND	ND	loss of cells, antigen status unknown
209	nCR	8 months	2 cells /uL blood	0	0.0852	loss of persistence, possible antigen negative tumor
257	SD	4 months	3 cells /uL blood	0.0125	115.7338	loss of persistence, antigen positive tumor
258	nCR	9 months	ND***	0.0001	0	cells present, very slight antigen detection
259	nCR	9 months	0****	0.0262	3.3309	loss of persistence, antigen positive tumor

*Day 360 not performed: day 180 = 0 and 0.3337, NY-ESO and Lage, respectively; day 420 = 0.1065 and 45.7363 (NY-ESO and Lage)

**day 180 value

***day 270 not available; day 42 =18 cells/uL and month 14 was negative

****Month 9 PBMC sample not received. Month 9 marrow negative. Month 6 PBMC <LOD

Supplementary Table 9. Results for the mixed-effects model of analyzing response profile: T-cell persistence in blood is inversely correlated with NY-ESO-1 levels in marrow.

Effect	Estimate&	Std. Err.	p-value
Intercept	4.55	1.68	0.014
NY-ESO-1	-47.11	18.85	0.022
LAGE-1	1.29	0.74	0.098

&adjusted for time

Supplementary Table 10. Estimated parameters and probabilities for a fitted joint model for longitudinal data and OS.

Variables in the model	Value	Std.Err.	p-value
NY-ESO-1	34.292	16.3954	0.037
LAGE-1	0.662	0.3444	0.055
CD138	1.659	1.0389	0.110

*all values were transformed using a square root transformation to decrease variability and to smooth distribution

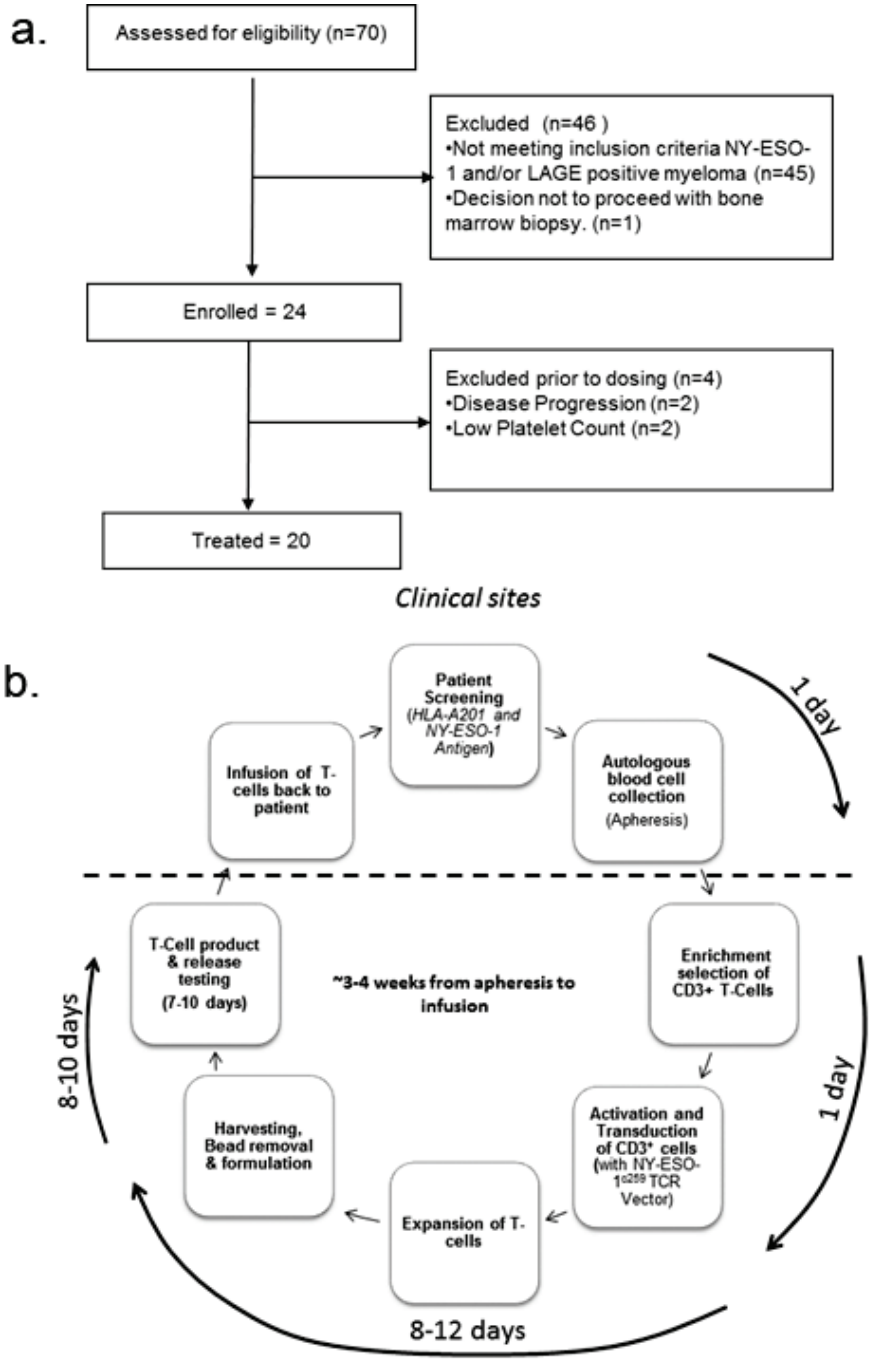
Supplementary Table 11. Estimated parameters and probabilities for a fitted joint model for longitudinal data and PFS.

Variables in the model	Value	Std. Err.	p-value
NY-ESO-1	52.355	31.223	0.094
LAGE-1	0.675	0.365	0.065
CD138	1.719	1.224	0.160

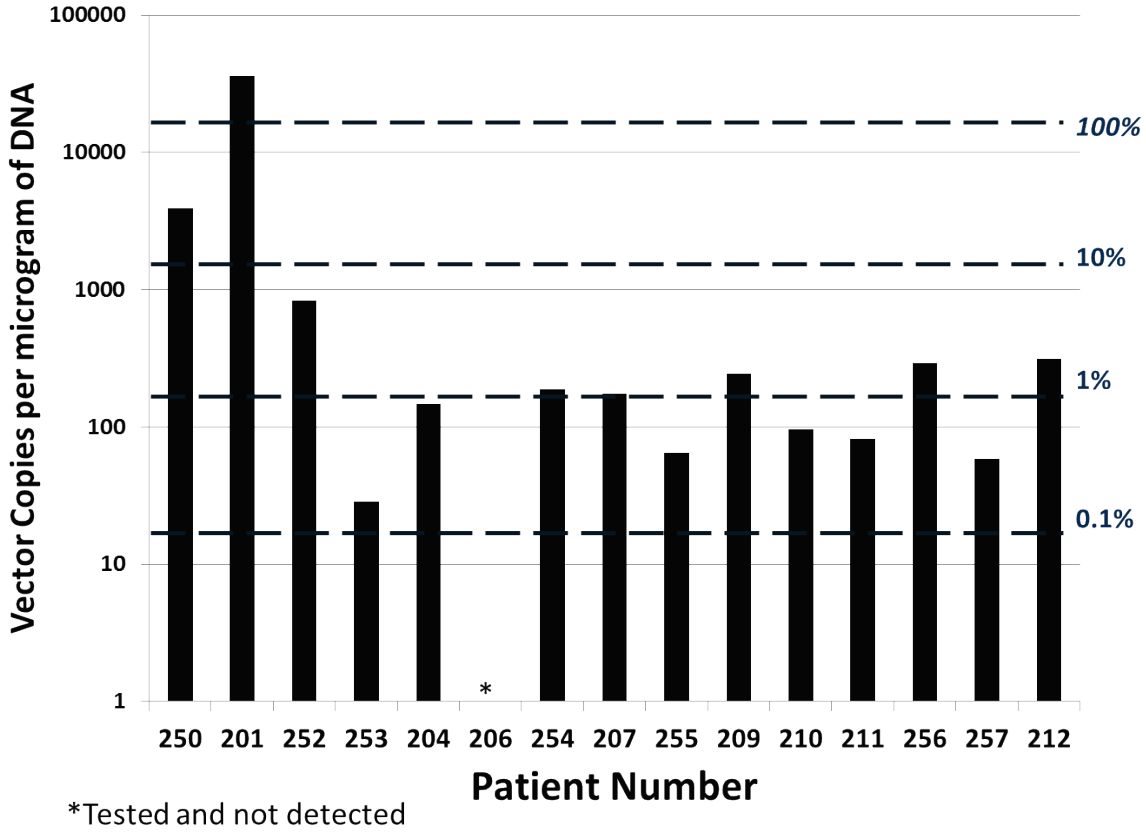
*all values were transformed using a square root transformation to decrease variability and to smooth distribution

Supplementary Figure 1. Consort statement and overview of manufacture.

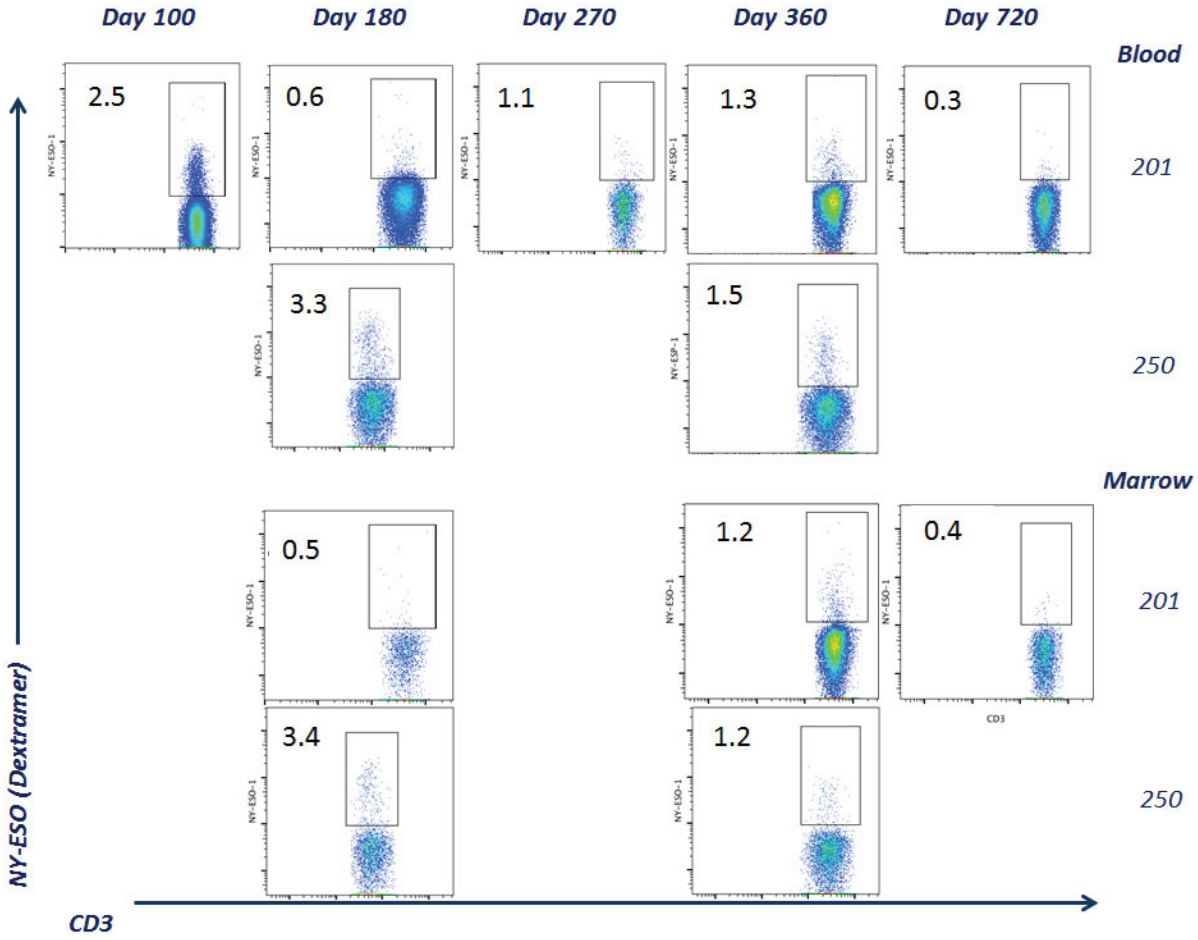
a. Overview of the number of HLA-A201 patients screened and who were entered onto the study. Approximately 1 patient, out of 3 screened, was eligible for enrollment based on HLA-A type and antigen expression (24/70 (34)). **b**. 10-12L apheresis product is collected at clinical sites and shipped overnight to a central manufacturing site. Cells were depleted of monocytes and CD25 positive cells, and activated with bead based artificial antigen presenting cells and gene modified with a lentiviral vector. Cells are expanded for 9-12 days and then frozen for release testing, which takes an additional 7-10 days. Patients are scheduled for ASCT once the cells are released back to the clinical site.



Supplementary Figure 2. Gene marked cells in marrow at day 100. A majority of patients underwent marrow collections at day 100 to assess response status, and the results of gene marking per microgram of DNA is represented in this panel. The percent of cells that the vector copies represent, assuming a single vector copy per cell, is shown by the dotted reference lines.



Supplementary Figure 3. Long term expression of NY-ESO TCR in blood and marrow. Patients 250 and 201 were evaluated for persistence of gene modified cells by flow cytometry. The top two rows show persistence in blood, and the bottom two show persistence in marrow. Mononuclear cells were isolated from blood and fresh marrow aspirates, and interrogated using CD3 antibody to detect T cells, and NY-ESO TCR specific dextramer reagent to detect gene modified cells. Gene marked cells are denoted by the box, and the number to the left of the box represents the overall percent positive cells from the CD3 positive fraction.



Supplementary Figure 4. Sequencing of TCR vβ Chains from Patient #253 marrow.

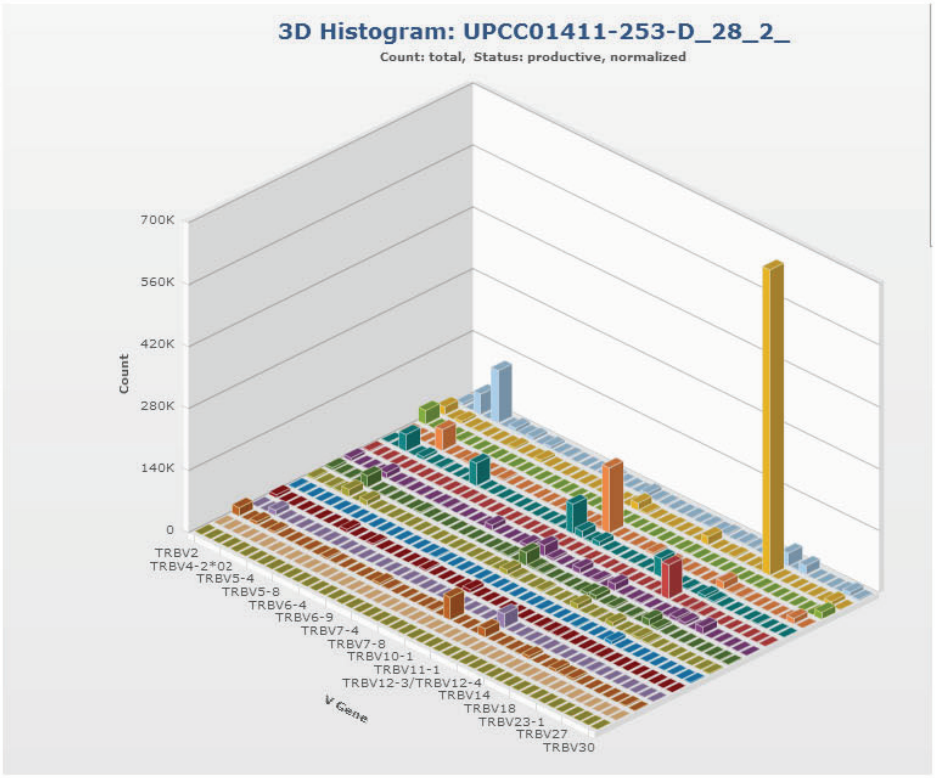
Histograms depicting the number of specific Vβ–Jβ gene segment combinations in the TCRβ chains are shown for the bone marrow of patient 253. **a**. marrow clonotype analysis from day 28 post second infusion. **b**. marrow clonotype analysis day 68 post second infusion.

Patient 253 had study treatment for aggressive relapse of kappa light chain secreting myeloma that occurred about 7 months after a first ASCT. Although she achieved a near-CR at day 100 post-ASCT #2 along with gene-modified T cells, her myeloma progressed by 6 months post-transplant. The study also allowed patients who had relapses of myeloma with NY-ESO-1/LAGE-1 antigen positive disease to receive a second infusion of gene-modified T-cells at up to a 5-fold higher dose after pre-conditioning with low-dose cyclophosphamide (1.5 g/m2) and 2 doses of bortezomib. Patient 253 had very strong antigen expression prior to the second infusion and also had a highly proliferative relapse of myeloma associated with hypercalcemia (12.7 mg/dl), an LDH level of 1076 (1.7 x ULN), transfusion-dependent pancytopenia, 10 grams/24 hours of Bence Jones kappa light chain excretion in the urine and a serum free kappa light chain level of 10,044 mg/L. Also, as shown in Fig 3c ("pre-T-cell panel"), the marrow was nearly 100% replaced by atypical CD138+ plasma cells many with plasmablastic features and all the metaphases showed 48-90 chromosomes with loss of 1p, 13q and deletion of 17p/p53 in 85% of the interphase cells by FISH analysis. Although the patient had previously progressed on lenalidomide maintenance and had no response to any bortezomib-containing regimens, she received the prescribed pre-conditioning using cyclophosphamide 1.5 g/m^2 and 2 doses of bortezomib followed by a infusion of 5 x 10^{10} total CD3+ T-cells (her second) which were 28.8 % transduced. By day +14 after second T-cell transfer, the absolute lymphocyte count (ALC) rose from 200 cells/μl to 4425 cells/μl and the hypercalcemia improved. By day +24, the calcium level was down to 10.5 mg/dl but the LDH level peaked at 1579 (2.6 x ULN) and the serum free kappa light chain spiked to 23,194 mg/L while the marrow showed a significant increase in marrow-infiltrating CD8+ T-cells (see figure 3c). Low-dose lenalidomide was started on day +24 at a dose of 10 mg per day and 8 days later at day +32, the ALC rose further to 8366 cells/μl while the serum free kappa light chain level decreased to 9500 mg/L, the 24 hour urinary Bence Jones protein decreased to 1.3 g/24 hours and the calcium level became normal at 9.1 mg/dl. By day +38, a marrow examination showed a dramatic increase in the marrow-infiltrating CD8+ T-cells to 70% while the marrow plasmacytosis was decreased to ~ 15% with extensive CD138+ myeloma cell necrosis (see Fig 3c). Over the next month, the patient experienced a return to a good quality of life and the 24 hour urinary excretion of Bence Jones protein decreased to as low 280 mg/24 hours.

While immunoassays showed early detection of the gene-marked cells (see Figure 2), during the period of major clinical response, the CD8+ T-cell compartment was comprised of 2 dominant

clonotypes that were not gene-marked. About 2 weeks after the point of maximal clinical response, the relapse of myeloma occurred coincident with a fall in the level of circulating lymphocytes.

A



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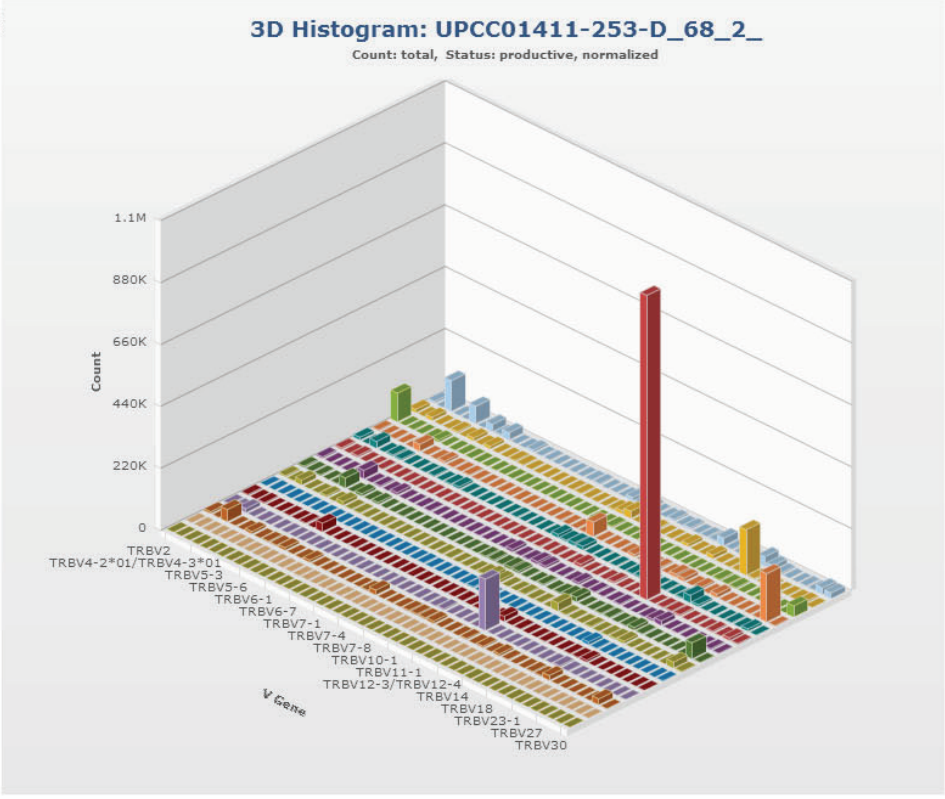
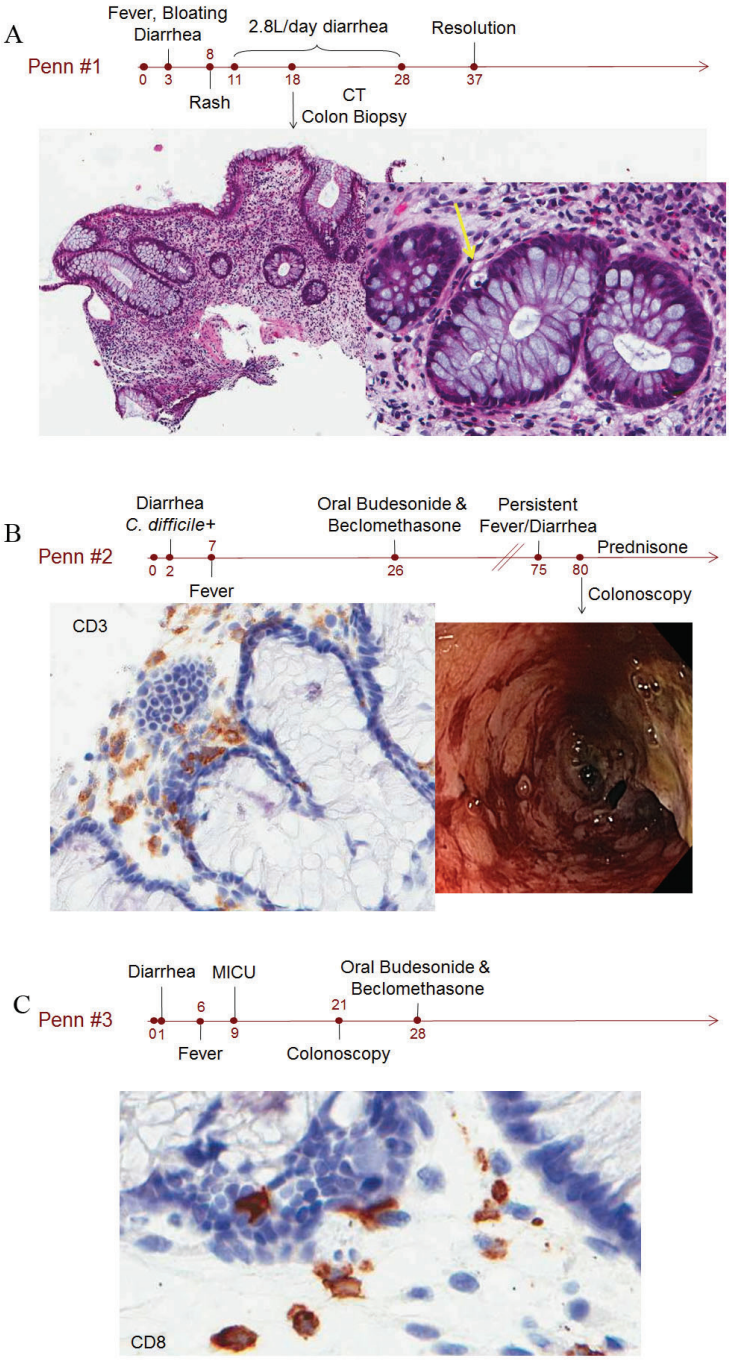
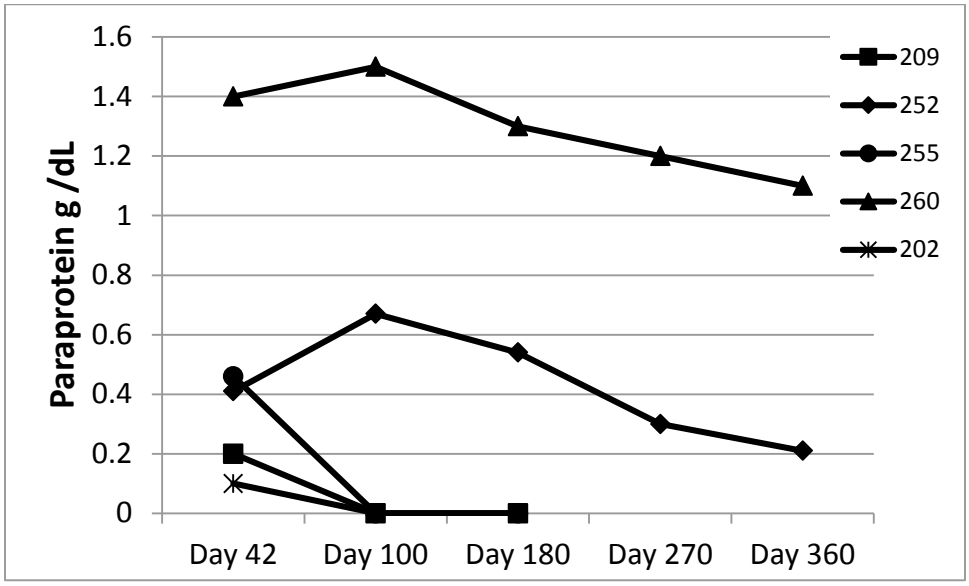


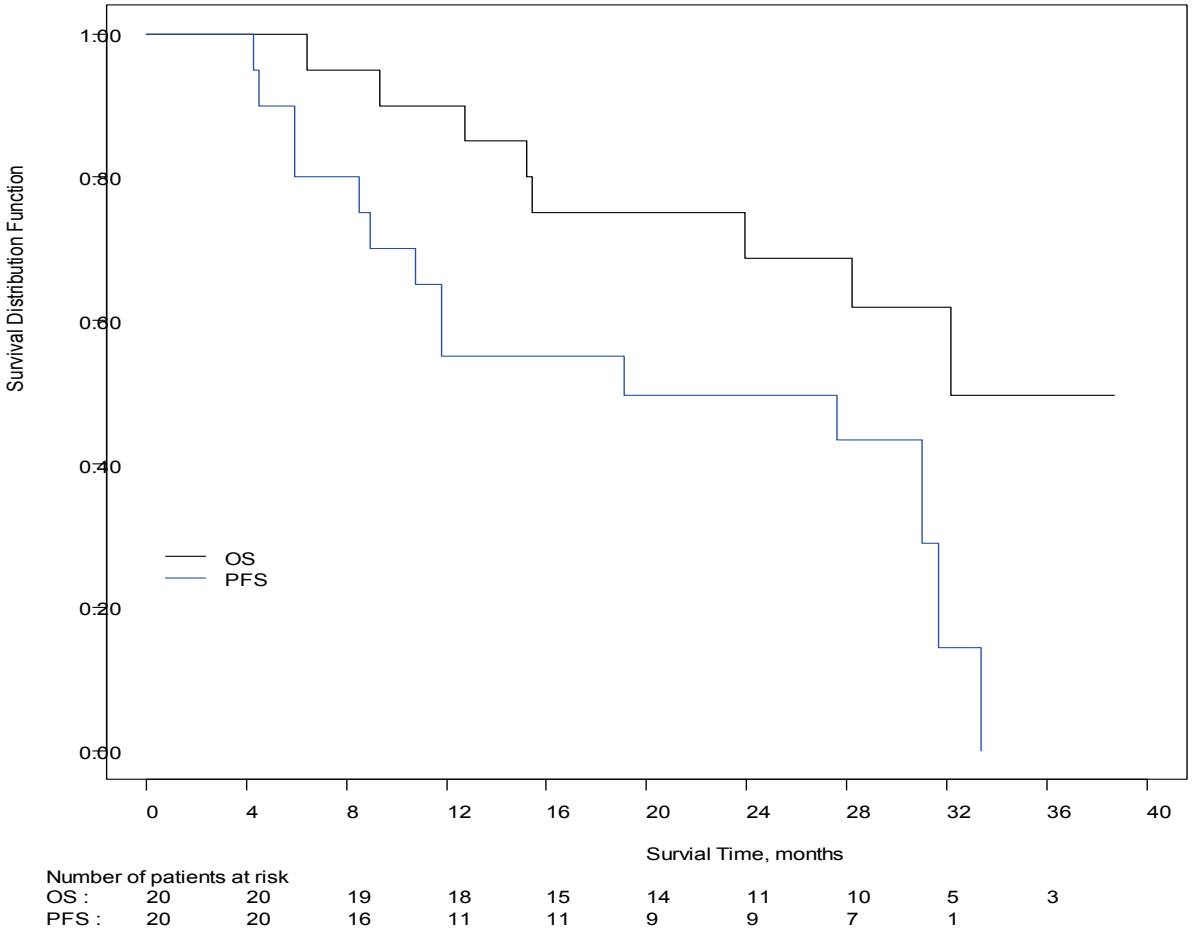


Supplementary Figure 5. Clinical presentation of autologous graft versus host disease. Three patients experiencing diarrhea post-transplant and T cell infusion, and which had colonic biopsies performed are shown. The pathology for each patient was consistent with autologous graft versus host disease, with signs of immune infiltrates and tissue damage. A timeline of events for each patient is shown along the top of each panel. The events either spontaneously resolved or resolved after topical steroids.

Supplementary Figure 6. Kinetics of paraprotein decline. 11 of the 20 patients enrolled on the study had measurable baseline levels of paraprotein. Of these 11, paraprotein levels decreased to undetectable levels by the initial tumor assessment at 6 weeks post-transplant in six patients (not shown). Of these six, patients 259 and 204 showed improvement in disease response as measured by immunofixation between the first and second tumor assessment period (Supplementary Table 1). 5 patients experienced a gradual decrease in paraprotein levels over time, as shown in this Figure. Note that lenalidomide maintenance was not started until day 100 post-transplant, and therefore improvements between day 42 and 100 are independent of this immune modulator. Patients 260 and 252 experienced declines after day 100 but did not receive lenalidomide maintenance (Supplementary Table 1). Notably patients 207 and 211 who had light chain only tumor, also experienced a gradual improvement of their disease between the first and second tumor assessment period (reference Supplementary Table 1).



Supplementary Figure 7. Overall Survival (OS) and Progression Free Survival, as estimated by the Kaplan-Meier method. Data as of April 1, 2015.



Supplementary Note. Case study description of patients #252 and #260, in support of immune surveillance against antigen positive tumour.

One patient is a 75 year old patient (252) who exhibited good persistence of gene-marked cells through day +360 along with a partial clinical response after 2nd ASCT and engineered T cell infusion per protocol. The monoclonal protein level fell from a pre-transplant value of 0.97 g/dl to a post-transplant value of 0.33 g/dl (day +180) corresponding to a decrease in marrow plasmacytosis from 10% to 4% (day +180). However, these residual myeloma cells were negative for NY-ESO-1 and LAGE-1 by PCR at days +42, +100, and +180 while gene-modified T-cells were detected in the blood and marrow at levels of 16-67 cells/mcl and 0.2-0.4% respectively during the same period of time. This patient remained in a stable PR at 2 years post-transplant while never having received lenalidomide maintenance due to post-transplant thrombocytopenia. A second patient (260) with active and treatment-refractory myeloma prior to transplant also had a minimal response to transplant and T-cell transfer (M-spike going from 1.7 g/dl to 1 g/dl) but this response was maintained for up to 9 months post-ASCT without any maintenance therapy and while follow up marrows at days 100 and 180 showed about 10% residual myeloma cells, tumor antigen expression was no longer detectable again suggesting selective elimination of the antigen-positive myeloma fraction which may have been responsible for the more aggressive clinical course that was observed before transplant. The patient has remained stable without myeloma progression at 1 year and 3 months post-ASCT.