

Abbreviated Title: Anti-MAGE-A3-DP4 TCR PBL

OBA: 1310-1253

IBC: RD-13-X-03

CC Protocol Number: P131408

NCI Protocol Number:

IRB Submission Date: 08/21/2013

Version Date:11/22/2013

PROTOCOL TITLE

Phase I/II Study of the Treatment of Metastatic Cancer that Expresses MAGE-A3 Using Lymphodepleting Conditioning Followed by Infusion of HLA-DP0401/0402 Restricted Anti-MAGE-A3 TCR-Gene Engineered Lymphocytes and Aldesleukin

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Prior Surgery Branch, NCI Efforts to Develop Effective Cell Transfer Therapies for Patients with Cancer (All First in Human)

- 1. Development of cell therapy for melanoma using tumor infiltrating lymphocytes (NEJM 319:1676, 1988).**
- 2. Introduction of foreign genes into humans; neomycin phosphotransferase gene into tumor infiltrating lymphocytes (NEJM 323:570, 1990).**
- 3. Use of cells genetically engineered to express T cell receptors (TCR) to treat melanoma (Science 314:126, 2006).**
- 4. Use of cells genetically engineered to express chimeric antigen receptors (CAR); B cell lymphomas and leukemias (Blood 116:4099, 2010).**
- 5. Use of cells genetically engineered to target cancer-testes antigens; NY-ESO-1 to target synovial cell sarcoma (J. Clin. Oncol. 29:917, 2011).**

Thus the current protocol to develop cell transfer therapies targeting MAGE-A3 represents a logical extension of prior Surgery Branch efforts.

Program for the Application of Cell Transfer Therapy to a Wide Variety of Human Cancers: Gene Modified Cells

Receptor	Type	Cancers	Status
MART-1	TCR	Melanoma	Closed
gp100	TCR	Melanoma	Closed
NY-ESO-1	TCR	Epithelial & Sarcomas	Accruing
CEA	TCR	Colorectal	Closed
CD19	CAR	Lymphomas	Accruing
VEGFR2	CAR	All cancers	Accruing
2G-1	TCR	Kidney	Accruing
IL-12	Cytokine	Adjuvant for all receptors	Accruing
MAGE-A3*	TCR	Epithelial	in development
EGFRvIII	CAR	Glioblastoma	Accruing
SSX-2	TCR	Epithelial	in development
Mesothelin	CAR	Pancreas & mesothelioma	Accruing
CSP4 (HMWAg)	CAR	Melanoma, Tnbreast, Panc	in development

*(MAGE-A3 TCRs; restricted by HLA-A2, A1, Cw7, DP4 – covers 80% of patients)

Cancer/Testes Antigens - Shared Tumor Specific Antigens

Expressed during fetal development

Restricted in their expression in adult normal tissues to germ cells

Up-regulated in 10-80% of cancers from multiple tissues

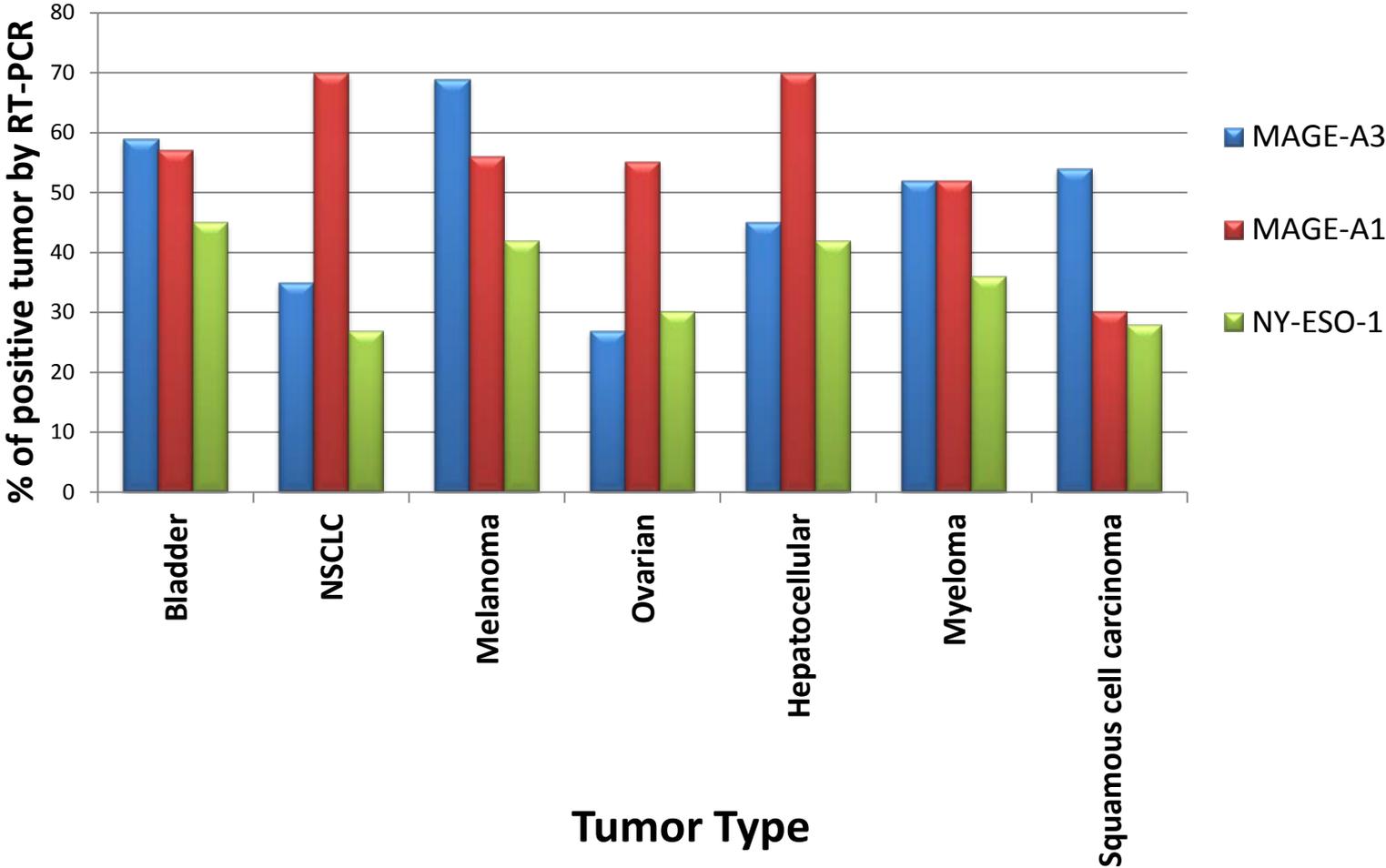
NY-ESO-1 Family

Small family of X-linked genes that includes NY-ESO-1 and LAGE-1

MAGE Family

Family of ~ 45 X-linked genes

Cancer/Testis Antigens Expressed in Multiple Tumor Types



Responses to Therapy with NY-ESO-1 TCR (5/1/13)

	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	19	6 (32%) (10**, 9+, 8, 5, 3, 3)	4 (21%) (50+, 49+, 25, 24+**)	10 (53%)
Synovial Cell Sarcoma	15	9 (60%) (31+**, 18*, 12**, 10, 8, 7, 5, 4, 3**)	1(7%) (5+)	10 (67%)

*treated twice

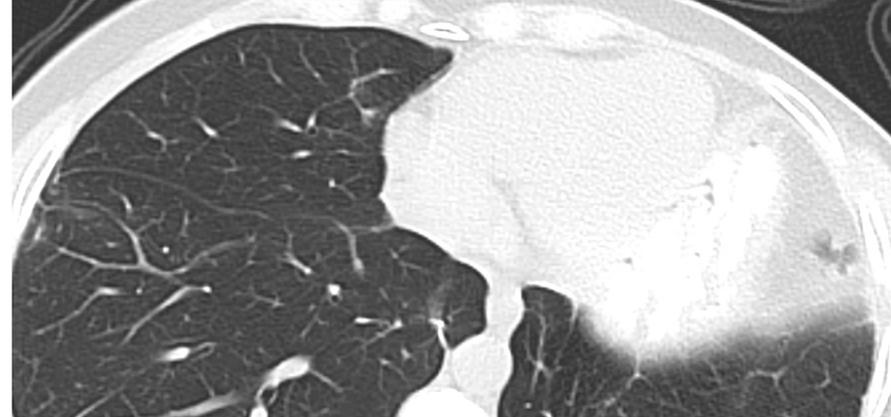
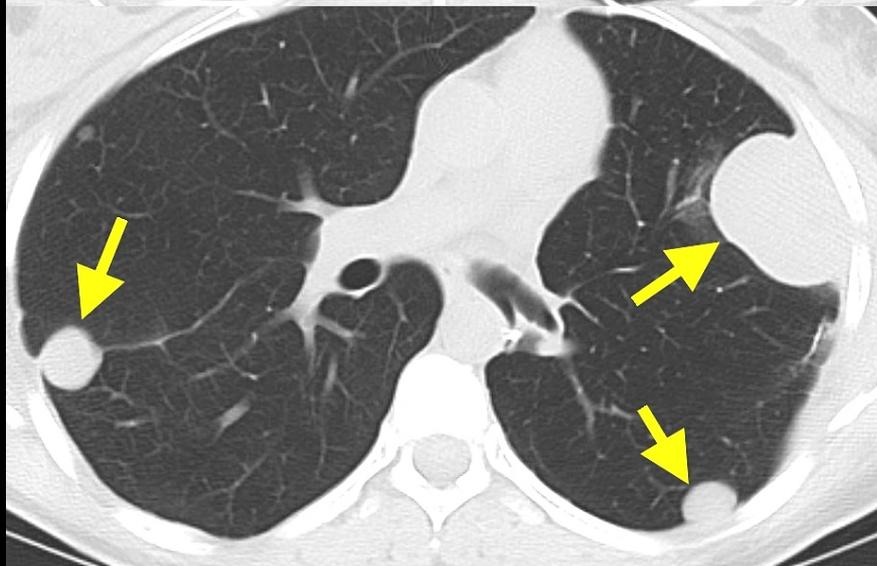
**plus ALVAC vaccine

(Robbins et al J Clin Oncol 29:917-924, 2011)

H.K.

Synovial
Sarcoma

ESO
TCR



Pre-Treatment

14 Months



Pre-Treatment



18 Months

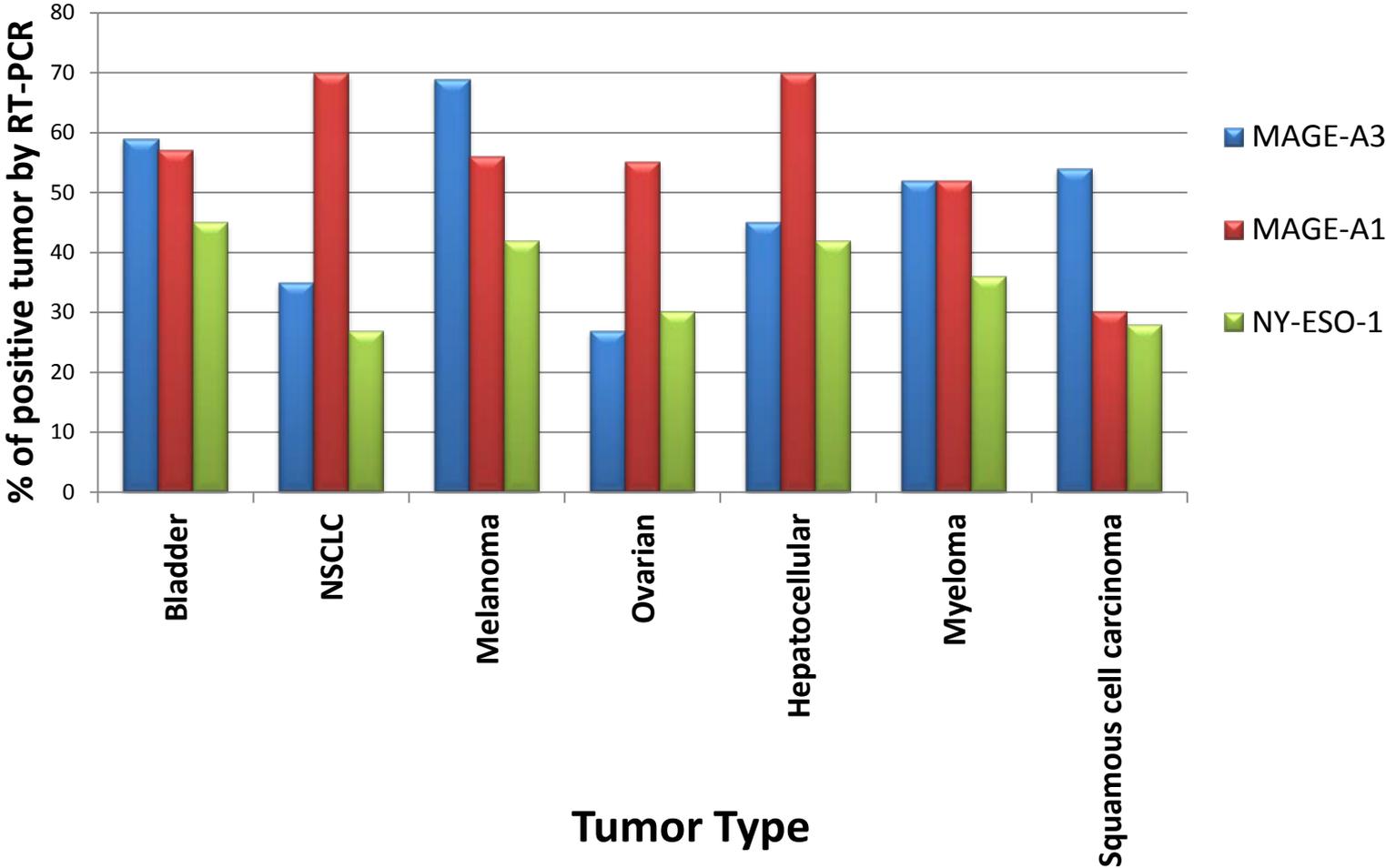


Pre-Treatment



18 Months

Cancer/Testis Antigens Expressed in Multiple Tumor Types



Cancer Regression and Neurological Toxicity Following Anti-MAGE-A3 TCR Gene Therapy

Richard A. Morgan, Nachimuthu Chinnasamy,* Daniel Abate-Daga,* Alena Gros,* Paul F. Robbins,* Zhili Zheng,* Mark E. Dudley,* Steven A. Feldman,* James C. Yang,* Richard M. Sherry,* Giao Q. Phan,* Marybeth S. Hughes,* Udai S. Kammula,* Akemi D. Miller,* Crystal J. Hessman,* Ashley A. Stewart,* Nicholas P. Restifo,* Martha M. Quezado,† Meghna Alimchandani,† Avi Z. Rosenberg,† Avindra Nath,‡ Tongguang Wang,‡ Bibiana Bielekova,‡ Simone C. Wuest,‡ Nirmala Akula,§ Francis J. McMahon,§ Susanne Wilde,|| Barbara Mosetter,|| Dolores J. Schendel,|| ¶ Carolyn M. Laurencot,* and Steven A. Rosenberg**

(J. Immunotherapy 36:133-151,2013)

Used a mouse derived T cell receptor (not subjected to negative selection in the thymus).

The HLA-A*0201 epitope targeted recognized a peptide in MAGE-A12 (MAGE A-12 was not previously known to be expressed in the brain).

No MAGE-A3 expression ever found in any adult normal tissue (except testes).

Identification of a Titin-Derived HLA-A1–Presented Peptide as a Cross-Reactive Target for Engineered MAGE A3–Directed T Cells

Brian J. Cameron,¹ Andrew B. Gerry,^{2*} Joseph Dukes,^{1*} Jane V. Harper,^{1*} Vivekanandan Kannan,¹ Frayne C. Bianchi,¹ Francis Grand,¹ Joanna E. Brewer,² Minnal Gupta,³ Gabriela Plesa,³ Giovanna Bossi,¹ Annelise Vuidepot,¹ Alex S. Powlesland,¹ Alison Legg,¹ Katherine J. Adams,² Alan D. Bennett,² Nicholas J. Pumphrey,² Daniel D. Williams,² Gwendolyn Binder-Scholl,² Irina Kulikovskaya,³ Bruce L. Levine,³ James L. Riley,⁴ Angel Varela-Rohena,⁴ Edward A. Stadtmauer,³ Aaron P. Rapoport,⁵ Gerald P. Linette,⁶ Carl H. June,³ Namir J. Hassan,¹ Michael Kalos,³ Bent K. Jakobsen^{1,2†}

ScienceTranslationalMedicine.org 7 August 2013

To produce high affinity MAGE-A3 A1-restricted TCRs, the authors introduced four amino acid substitutions into the TCR α/β chain CDR regions from a human TCR (thus no negative selection in the thymus)

Two patients treated with T cells engineered to express the this modified TCR died due to acute cardiac failure within 5 days of T cell administration.

Post-clinical trial analyses that identified a Titin-derived peptide presented by HLA-A1 as the likely cause of the in vivo toxicity.

The unmodified MAGE-A3 TCR did not recognize this peptide.

Rationale for Targeting the HLA-DP0401/DP0402 Class II Restricted Epitope of MAGE-A3

CD4⁺ T cells can be highly effective in eradicating murine tumors.

CD4⁺ clone reactive with an HLA-DP0401 restricted antigen mediated cancer regression in a patient with metastatic melanoma.

TCR 6F9 recognizing the MAGE-A3:243-258 epitope restricted by HLA-DP0401 was isolated from a patient immunized against MAGE-A3.

Replacement of human with mouse constant region enhanced recognition of MAGE-A3.

HLA-DP0401: 70% of caucasians

HLA-DP0402: 20% of caucasians

Specificity of HIA-DP0401/02 Class II restricted TCR 6F9mc

Highly specific recognition of MAGE-A3+, DP0401+ tumors

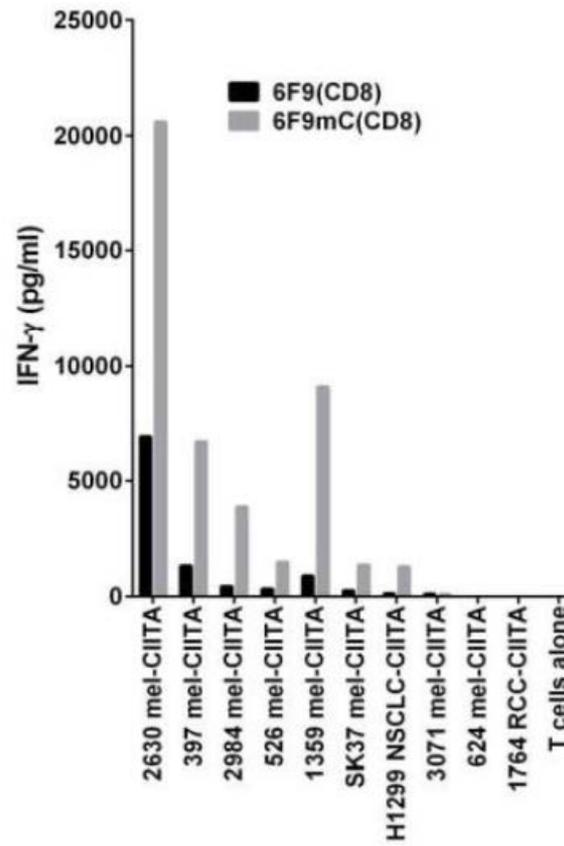
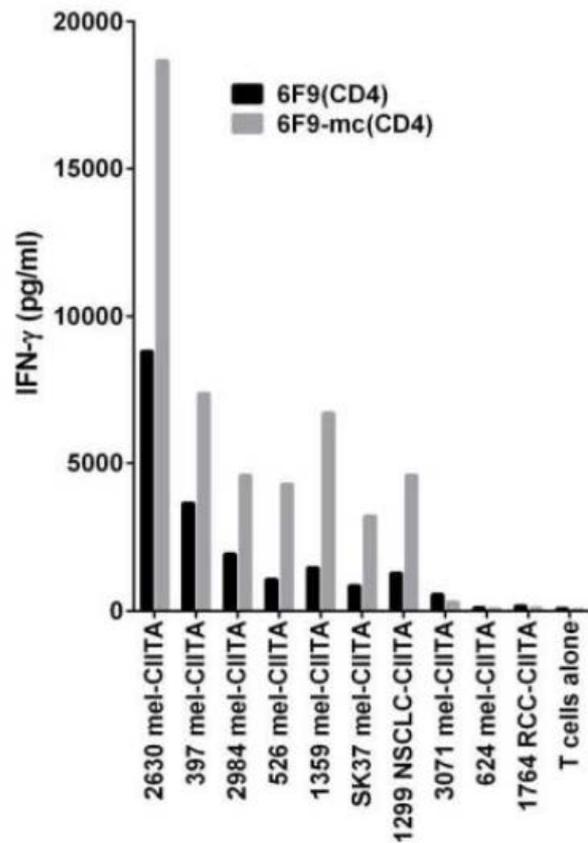
Recognition of MAGE-A6 peptide (differs by one α from MAGE-A3:243-258 epitope).

No MAGE-A3 or MAGE-A6 expression on any normal tissue except testes.

No recognition of any other MAGE epitopes (differ between 2-5 α).

BLAST search of NCBI database revealed most closely related epitope (5 α difference from MAGE-A3 epitope): not recognized

Specificity of the MAGE-A3/ DP0401/02 T Cell Receptor



MAGE-A3+/DP*0401+

MAGE-A3+/DP*0402+

MAGE-A3+/DP*04-

MAGE-A3-/DP*0401+

2630 mel-CIITA

3071 mel-CIITA

624 mel-CIITA

1764 RCC-CIITA

397 mel-CIITA

2984 mel-CIITA

526 mel-CIITA

1359 mel-CIITA

SK37-mel-CIITA

H1299 NSCLC-CIITA

Specificity of the MAGE-A3/ DP0401/02 T Cell Receptor

Gene (position)	Amino Acid Sequence	PBMC-1* transduced with:			PBMC-2 transduced with:			Predicted affinity(nM)
		6F9	6F9mc	None	6F9	6F9mc	None	
		IFN- γ (pg/ml)						
MAGE-A3:243-258	KKLLTQH FVQ ENYLEY	10,220	15,210	33	10,350	17,520	45	3
MAGE-A3:243-256	KKLLTQH FVQ ENYL	1,018	1,815	72	1,670	2,490	78	323
MAGE-A3:243-255	KKLLTQH FVQ ENY	76	137	29	111	117	71	378
MAGE-A3:243-254	KKLLTQH FVQ EN	28	0	67	30	39	78	466
MAGE-A3:243-253	KKLLTQH FVQ E	0	40	38	30	45	90	2444
MAGE-A3:245-258	LLTQH FVQ ENYLEY	9,290	14,970	84	8,920	17,820	74	3
MAGE-A3:246-258	LTQH FVQ ENYLEY	7,140	12,700	56	9,200	16,170	76	3
MAGE-A3:247-258	TQH FVQ ENYLEY	6,710	10,600	30	6,810	13,280	41	3
MAGE-A3:248-258	QH FVQ ENYLEY	6,220	9,000	52	7,400	8,700	56	4
MAGE-A3:249-258	HFV Q ENYLEY	669	1,643	57	922	2,034	66	5
MAGE-A6:248-258	QYFV Q ENYLEY	6,440	11,800	54	13,200	8,370	127	3
MAGE-A2/A12:248-258	QDLV Q ENYLEY	33	66	49	37	56	65	59
MAGE-A4/A9:249-259	QD WVQ ENYLEY	0	23	32	22	26	62	92
MAGE-A8:251-261	QEWV Q ENYLEY	43	58	79	39	41	55	87
MAGE-A1/B4:241-251	QDLV Q EKYLEY	129	126	55	108	84	53	16
MAGE-B2:250-260	KDLV Q EKYLEY	0	0	43	7	20	38	16
MAGE-B10:250-260	KDLV K ENYLEY	22	18	69	28	34	66	105
MAGE-B16:252-262	KDFV K EKYLEY	0	27	16	11	28	42	3
MAGE-C1:113-123	KV WVQ EHYLEY	9	0	30	25	27	30	35
MAGE-D4:300-315	RKLITDDFV KQ KYLEY	193	234	81	194	268	80	6
MAGE-D2:413-428	KKLITDEFV KQ KYLDY	82	43	56	226	223	71	8
MAGE-L2:582-597	KKLITEV FVRQ KYLEY	45	56	58	78	107	114	6
MAGE-G1:220-235	KKLIT E DFV RQ RYLEY	0	29	68	25	33	62	3
Necdin:237-247	EEFV Q MNYLKY	0	22	59	21	32	83	13
No peptide		0	5	58	15	25	59	

Objectives

Primary objectives:

- Determine a safe dose of the administration of autologous CD4 cells transduced with an anti-MAGE-A3-DP0401/0402 restricted (MAGE-A3-DP4) TCR and aldesleukin to patients following a nonmyeloablative but lymphoid depleting preparative regimen.
- Determine if this approach will result in objective tumor regression in patients with metastatic cancer expressing MAGE-A3-DP4.
- Determine the toxicity profile of this treatment regimen.

Secondary Objective:

- Determine the in vivo survival of TCR gene-engineered cells.

Eligibility

Patients who are HLA-DP0401/0402 positive and 18 years of age or older must have

- Metastatic cancer whose tumors express the MAGE-A3-DP4 antigen;
- Previously received and have been a non-responder to or recurred following at least one first line treatment for metastatic disease;

Patients may not have:

- Contraindications for high dose aldesleukin administration.

Protocol Design

**PBMC transduced with retroviral vector encoding the anti-MAGE-A3
HLA-A-DP0401/0402 TCR**

cohort 1: metastatic melanoma

cohort 2: other solid metastatic cancers

**Patients receive the SB standard non-myeloablative preparative
regimen followed by cell infusion and IL-2**

Phase I dose escalation: half-log increments from $1e7$ to $1e11$

Phase I Dose Escalation: DP0401

Restricted anti-MAGE-A3 TCR

(1 patient in each dose cohort unless a patient experiences a dose limiting toxicity; 2 week delay between cohort escalation).

Cohort 1	10⁷ cells MAGE-A3-DP0401 restricted transduced cells
Cohort 2	3 x 10⁷ cells
Cohort 3	10⁸ cells
Cohort 4	3 x 10⁸ cells
Cohort 5	10⁹ cells
Cohort 6	3 x 10⁹ cells
Cohort 7	10¹⁰ cells
Cohort 8	3 x 10¹⁰ cells
Cohort 9	10¹¹ cells

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Phase II design

if < 2 responses of 21: Stop

if ≥ 2 responses of 21: enter 41

Rules out 5% response rate in favor of a 20% response rate

Anti MAGE-A3 DP0401/02 Restricted T-Cell Receptor in this Protocol

Unmodified TCR obtained from a human immunized against MAGE-A3 (thus subjected to negative selection in human thymus)

As shown in the protocol, this receptor:

recognizes MAGE-A3 HLA-DP0401/02 tumors with high specificity

does not recognize any of the other MAGE family members except MAGE-A6; MAGE-A3 and MAGE-A6 not detected in any normal human tissue

protein blast search against the human Refseq protein data base reveals no close sequences

GMP quality anti MAGE-A3 HLA –DP0401/02 receptor encoded in a gammaretrovirus produced in the Surgery Branch and approved by the FDA

MAGE-A3 HLA-DP0401/02 TCR Protocol

Date approved

August 20, 2013

Scientific Review Board

September 13, 2013

IRB (minor stipulations)

November 1, 2013

FDA

November 6, 2013

Institutional Biosafety Committee

(Now RAC review necessary before we can submit for final IRB approval)