Affinity Optimized NY-ESO-1 and MAGE-A3/6 Specific T Cells for Melanoma

Protocol #1007-1057

Phase I Study to Assess the Safety and Activity of Enhanced TCR Transduced Autologous T Cells Against Cancer-Testis Antigens In Metastatic Melanoma

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Penn Medicine
Abramson Cancer Center

Adaptimmune
Transforming T Cell Therapy

Protocol 1057: Investigative Team

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Protocol 1057: Investigative Team

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Outline

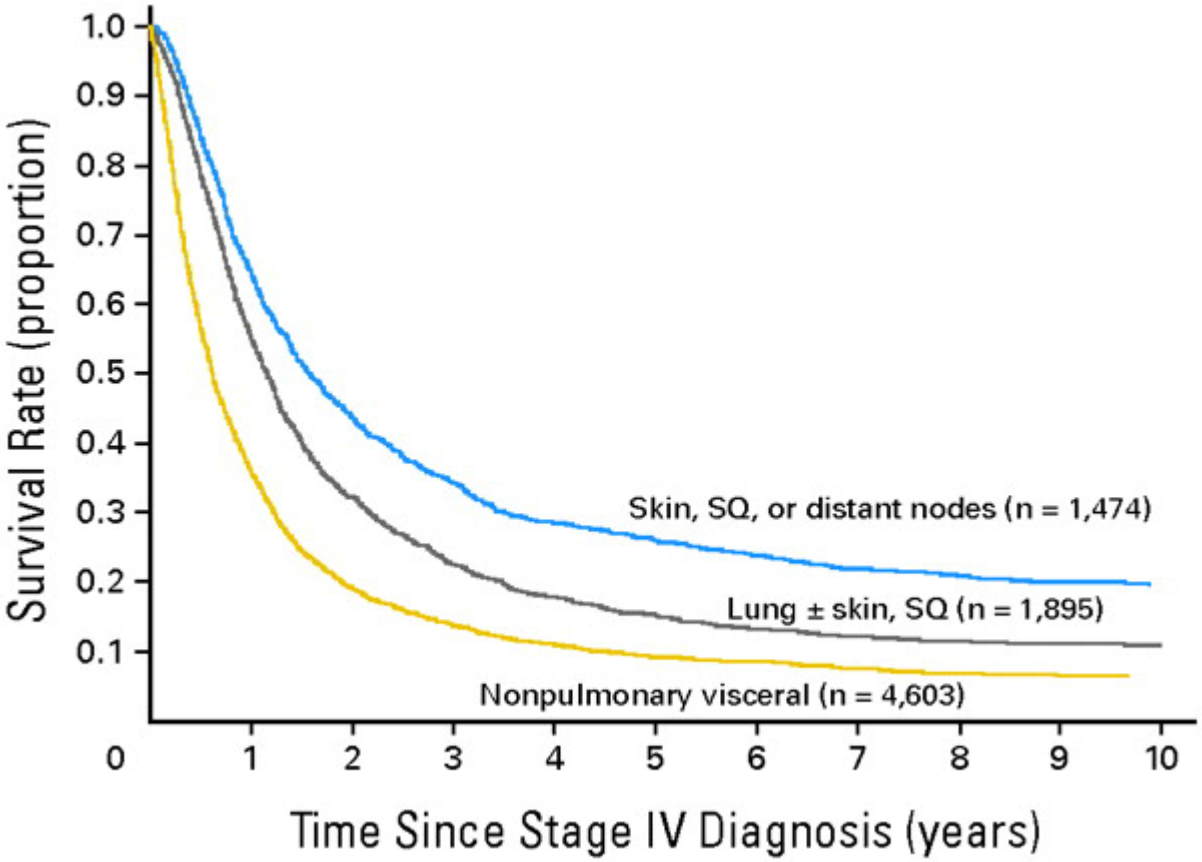
- Melanoma background
- Design and testing of affinity enhanced TCRs
- Pre-clinical testing
- Protocol design and safety features to minimize toxicity and maximize scientific rigor

Cutaneous Melanoma

- 68,130 new cases (estimated) in 2010
  - Most patients are cured with surgery alone.
- 20% relapse and develop metastatic disease involving lymph nodes (stage III) or distant sites (stage IV)
  - Lungs, liver, and brain are most common sites.
- Treatment is surgery → Interferon (stage III) Dacarbazine or IL-2 (stage IV)
  - Stage IV is incurable with expected survival of 6-10 mo.
- Treatment options include clinical trials evaluating new targeted agents or immunotherapy

Melanoma Survival: unmet medical need

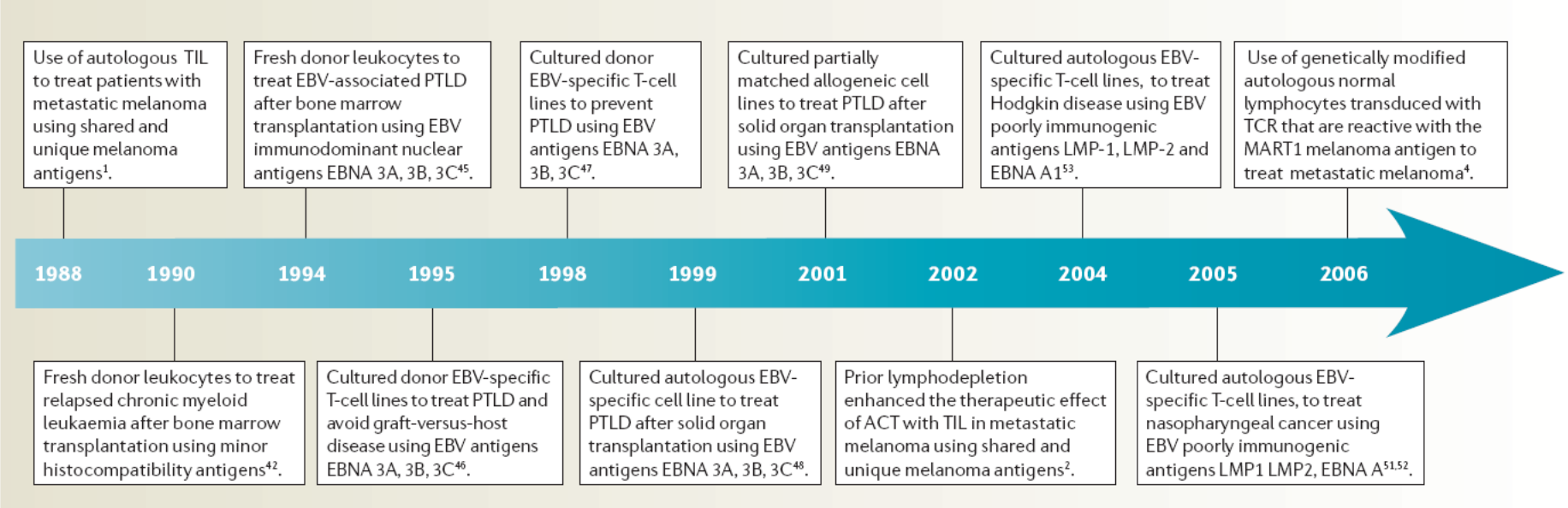
Stage IV



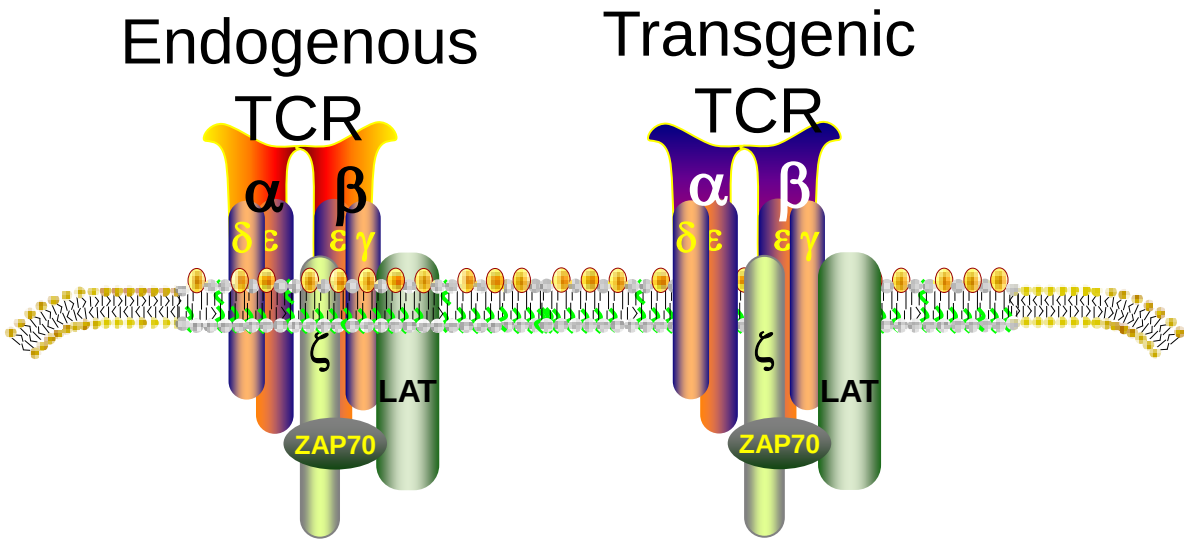
J Clin Oncol. 27:6199, 2009

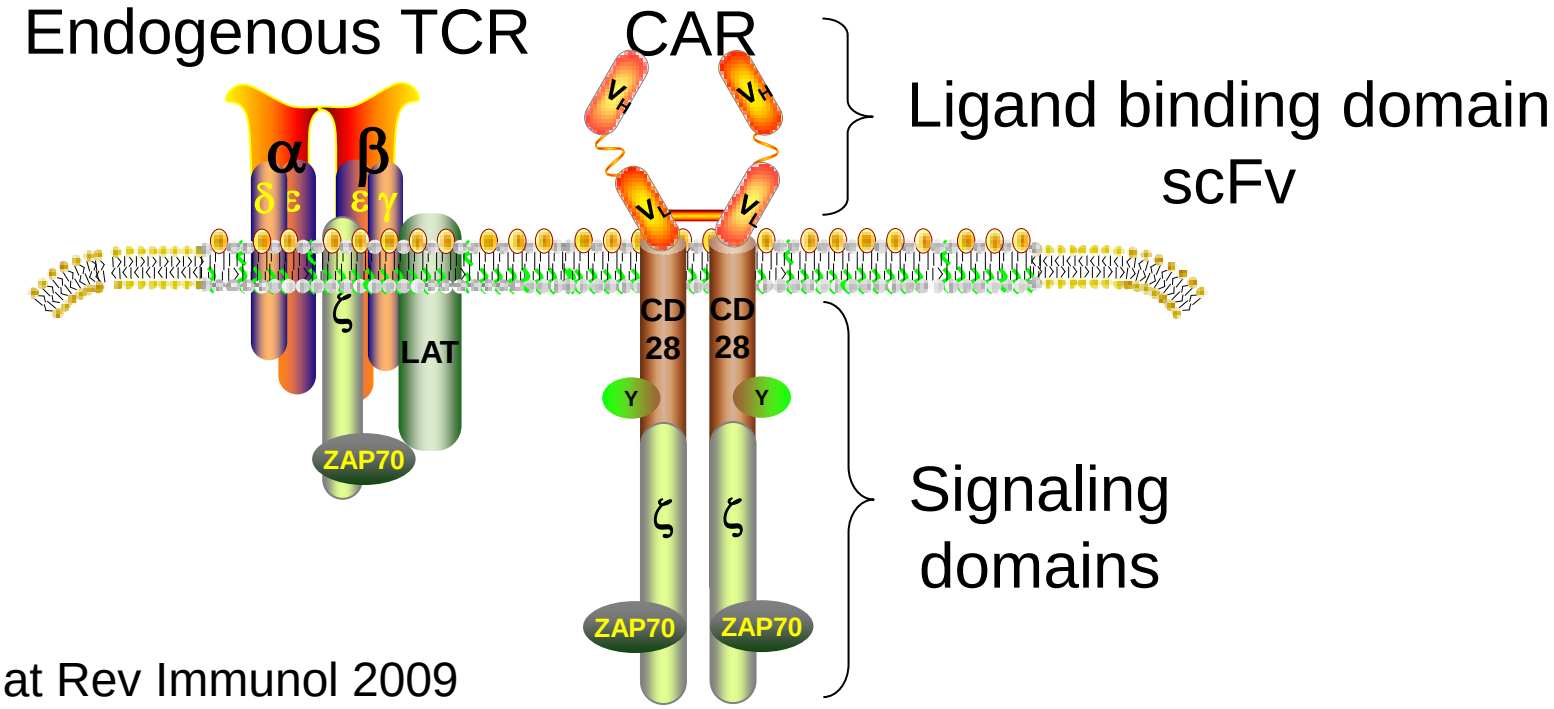
Adoptive T cell therapy

- Tumor infiltrating lymphocytes (TIL)
- T cell clones
- TCR transduced T cells (TCR gene therapy)



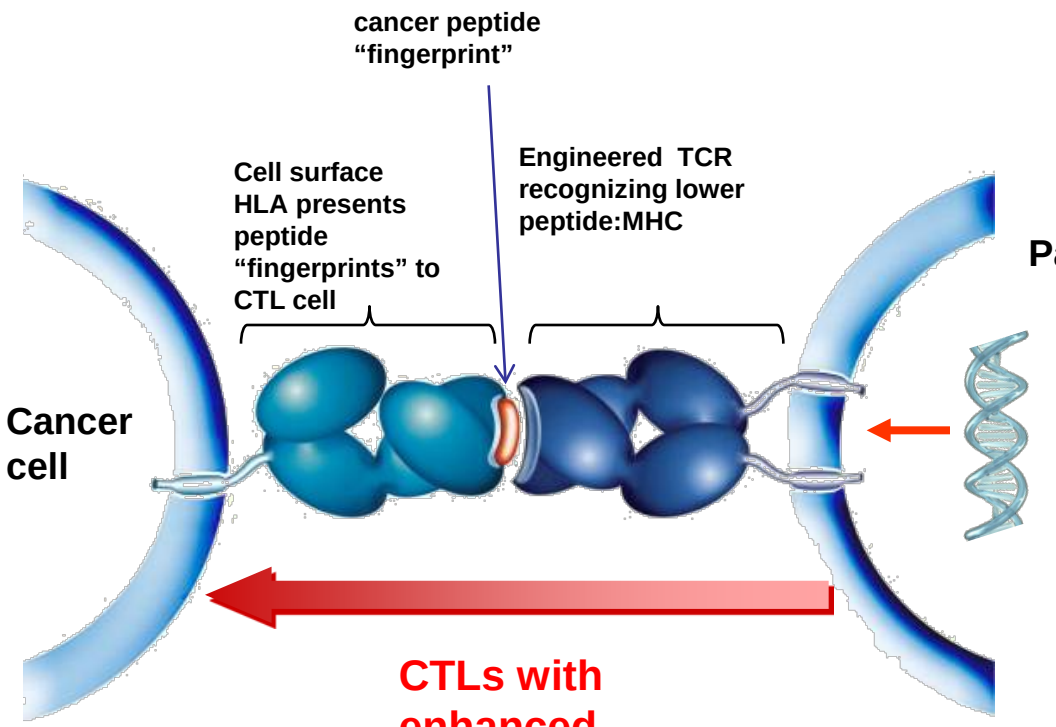
Therapy with genetically retargeted T cells





June et al, Nat Rev Immunol 2009

Rationale for therapy with natural and genetically retargeted CTLs



Patient’s natural CTL

DNA encoding the new engineered version of the killer T cell receptor is given to the patient’s own isolated killer T cells

CTLs with enhanced TCRs kill cancer cells expressing low density p:MHC targets

Rationale to target cancer testis antigens

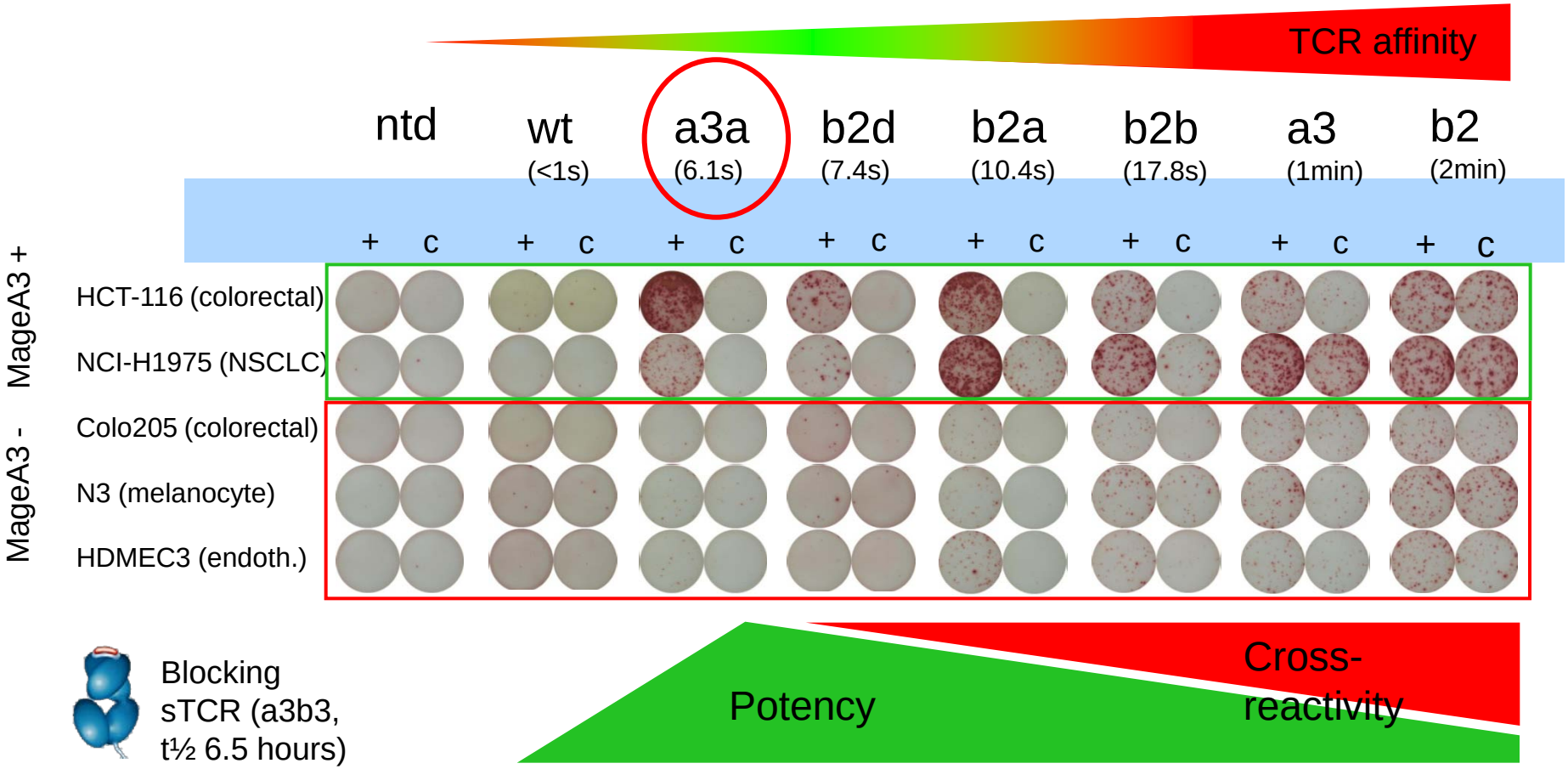
1. In adults, expression restricted to tumors and gametogenic tissues

	MageA3	NY-ESO-1
Melanoma	36%	34%
Lung cancer	47%	16%
Myeloma	70%	>70%
Ovarian cancer	17%	25%

2. Genes frequently on chromosome X (CT-X, non-X CT)
3. Multigene families
4. Coordinated expression of multiple CT
5. Heterogenous protein expression
6. Higher expression correlated with worse outcome (progression, metastasis)
7. Biologic function ?
8. Regulation: methylation, HDAC inhibitors, specific transcription factors, methyl-CpG binding proteins
9. Immunogenic:
   - Phase III cancer vaccine trials underway in lung cancer and melanoma targeting MAGE A3
   - Safety and antitumor effects observed with NY-ES01^{c259} TCR (NCT00670748)

http://www.cta.lncc.br/

T Cells Transduced with MAGE A3/HLA-A1 Specific TCRs



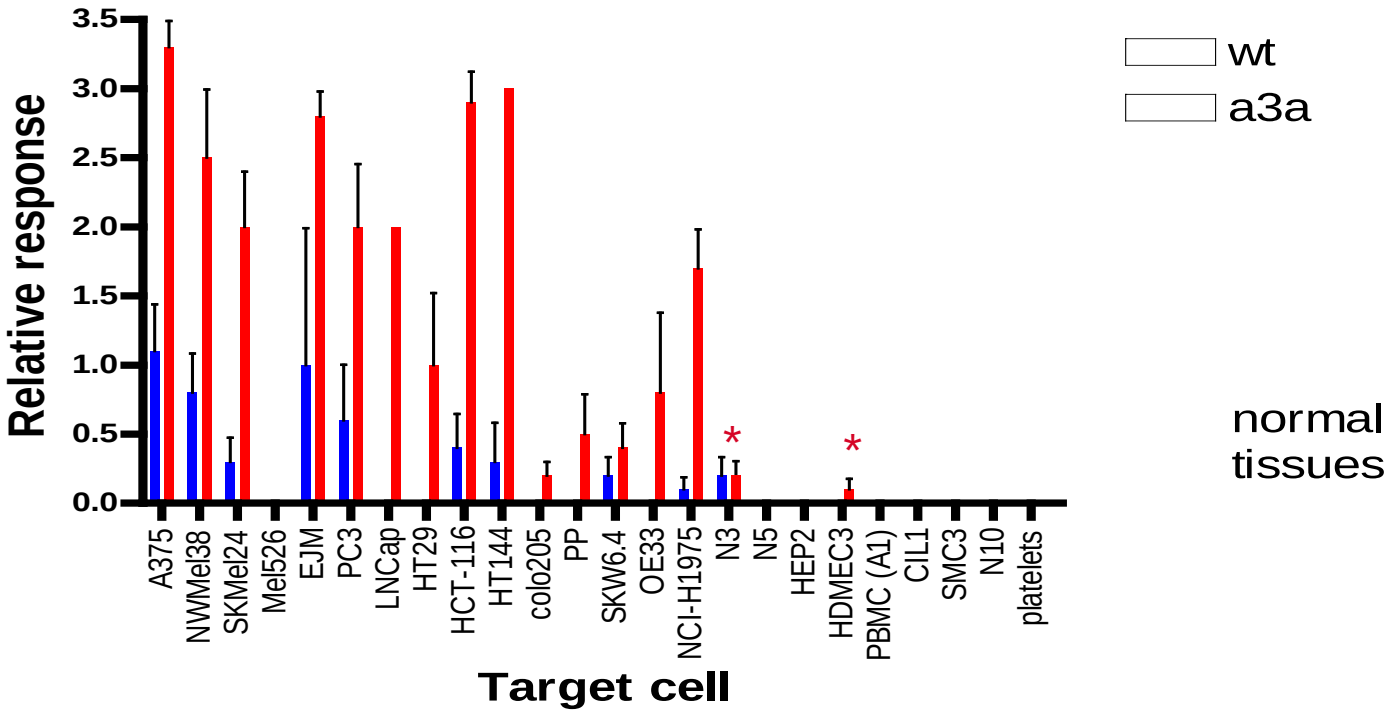
+ = Target cells & TCR transduced T cells

c = Target cells, TCR transduced T cells & pico-molar affinity, soluble TCR masking antigen

MAGE A3^{a3a} TCR

- Recognizes MAGE peptide EVDPIGHLY/HLA-A*0101 complex
- This TCR has 4 α-chain mutations and a wild type β-chain
- T cells transduced with this TCR do not react with normal cells

IFN-γ response of MAGE-A3 TCR transduced T cells to various tumor and normal cell lines



* N3 cells: response shown above only present in 3/8 assays

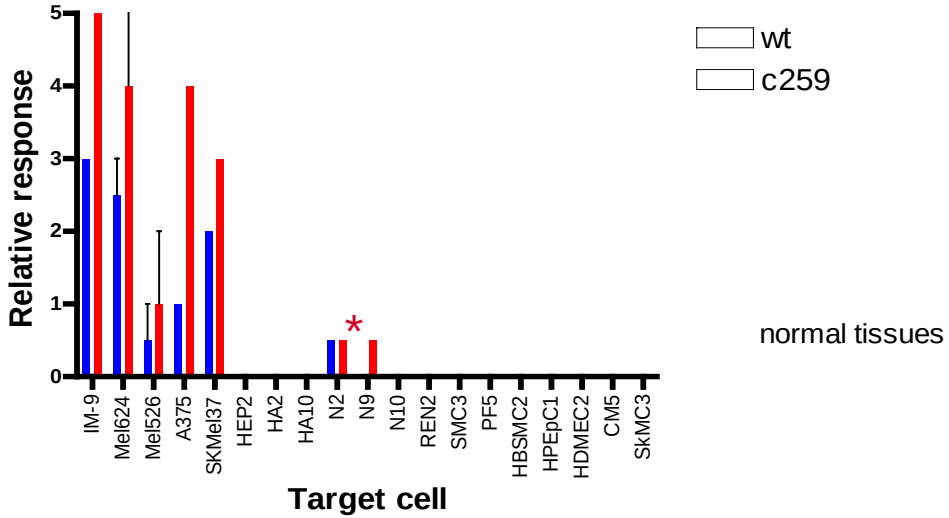
* HDMEC3 cells: response shown above only present in 1/7 assays

Generation of optimized NY-ESO-1 TCR (HLA-A2)

- Derivation of TCR described (Robbins et al, J Immunol, 2008).
  - 2 mutations in the CDR3α chain.

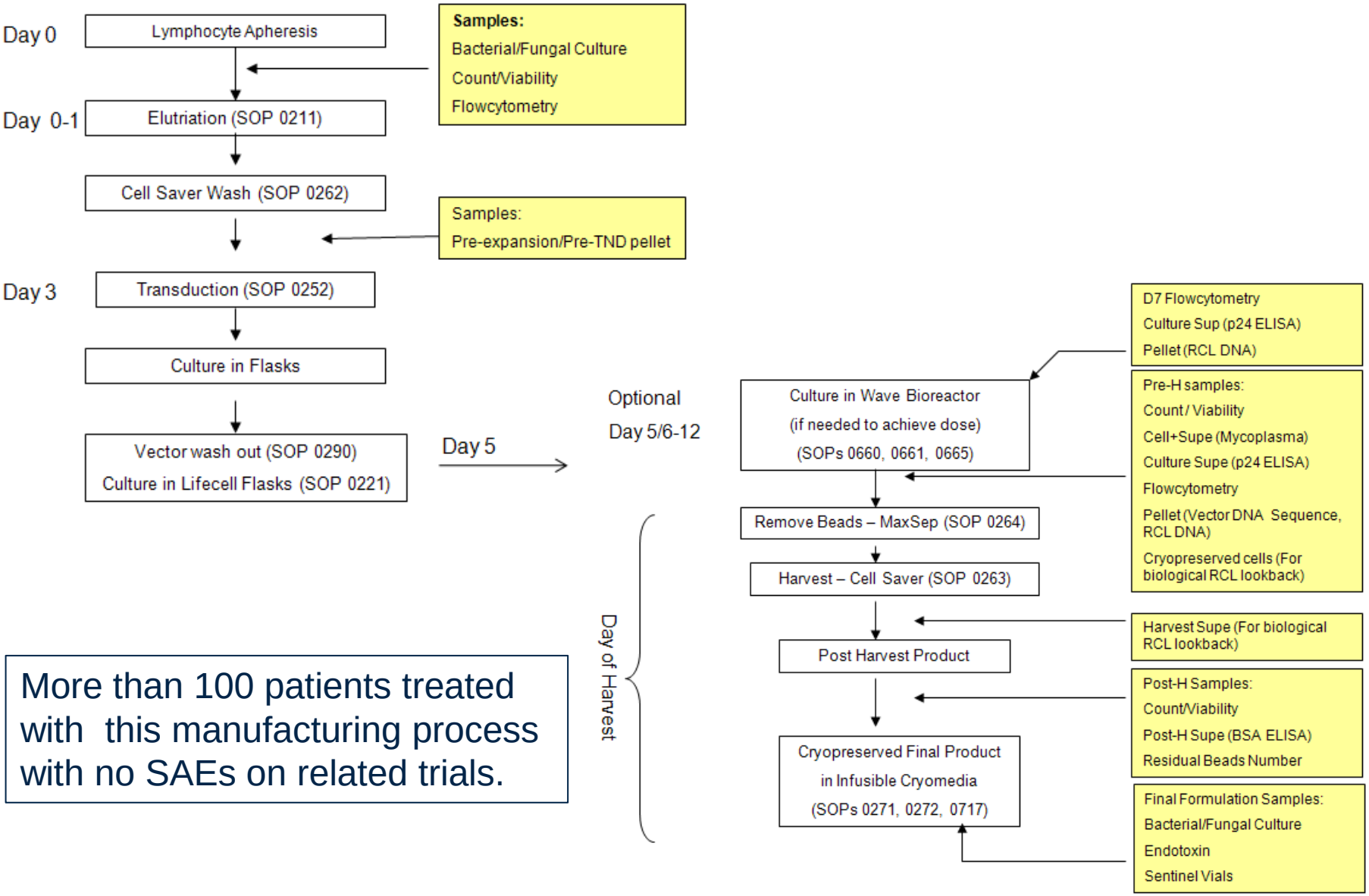
- NY-ESO-1^{c259} TCR has been tested in the clinic (NCT00670748)
  - up to 1.3e11 TCR transduced T cells administered with no on- or off-target toxicities. No MTD identified.
  - tumor regression in 5/11 melanoma and 4/6 sarcoma patients (Robbins et al, AACR 2010) .

Binding kinetics of 1G4 sTCRs		
Clone	$T_{1/2}$	KD
aWT/bWT ('wt')	2.2 sec	9.30μM
a259/bWT ('c259')	19.0 sec	730nM



* N2: similar to observation in MAGE-A3^{a3a} TCR but no off target effects seen in patients
N9: results shown in 3/3 experiments no activity in LDH killing assay

Cell manufacturing process: CVPF at UPenn



More than 100 patients treated with this manufacturing process with no SAEs on related trials.

Protocol 1057

Phase I Study to Assess the Safety and Activity of Enhanced TCR Transduced Autologous T Cells Against Cancer-Testis Antigens In Metastatic Melanoma

Rationale

TIL therapy is currently the most effective therapy for metastatic melanoma, however most patients are <u>not</u> eligible because of technical limitations regarding TIL manufacturing

Peripheral blood derived, TCR-modified T cells have potential advantages over TIL therapy

- **Rapid generation of antigen specific T-cells**
- **Eliminate the need for surgery**
- **Generate antigen specificities with potency not found naturally in patients**

Pilot TCR trials with TCRs directed against MART-1 and gp100 melanocyte differentiation antigens were promising at the NCI

- **Objective response rate of 30% observed in a recent trial carried out with a MART-1 reactive TCR (Johnson et al, Blood 2009)**
- **TCRs specific for cancer testis antigens have the potential to decrease toxicity and increase antitumor efficacy.**

Study Design

Enroll

- **HLA-A*0201+ patients with NY-ESO-1+ tumors**
- **HLA-A*0101+ patients with MAGE A3/6+ tumors**

Apheresis

T cells activated using anti-CD3/CD28 beads

T cells transduced with lentivirus

- **NY-ESO-1^{c259} TCR**
- **MAGE^{a3a} TCR**

Pre-conditioning will be done using cyclophosphamide @ 60mg/kg x 2d

T cells administered using split dose infusion strategy

- **30% on day +5**
- **70% on day +6**

Schema

- Histologically confirmed melanoma, stage III/IV unresectable
- Age ≥ 18
- ECOG 0 or 1
- HLA-A*0101 or HLA-A*0201 positive
- One prior systemic therapy is allowed
- Written informed consent

n=6 pts for NY-ESO-1 TCR
n=6 pts for MAGE-A3/6 TCR

↓

- Screen tumor for cancer-testis antigen expression

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- Apheresis → ship to the University of Pennsylvania for manufacturing

↓

- Screening evaluation, including imaging studies and blood work

✓ If eligible and the transduced T cell product passes QC, may proceed to the treatment phase →

Conditioning Regimen (day 1 and 2)

- Mesna 1500 mg/m2 iv over 15 min prior to and 4 hr after CTX
- Cyclophosphamide 60 mg/kg iv over 2 hr qd, day 1 and 2

↓

T Cell Therapy (day 5,6)

- Day 5 30% cell product over 15 min
- Day 6 70% cell product over 15 min

- Serum for cytokine analysis 20 min post-infusion (days 5,6)
- Immune Monitoring (at specified time points)

Total
Dose: 1x10e10 T cells

↓

Follow up (one year)

- Weekly H&P with CBC, CMP and study labs (12 weeks)
- CT imaging at 4,8,12 weeks, then every 2 mo
- Study apheresis at 4 and 8 weeks
- Study Biopsy at 4-8 weeks

Treatment Plan

1. Only HLA-A1 and HLA-A2 positive patients are offered participation and informed consent is obtained.
2. Fresh tumor is sent to Penn for testing of NY-ESO/MAGE A3 by qPCR; if fresh tumor is not available, archived fna (fine needle aspirate) will be used for IHC. If negative→ screen failure.
3. If positive, apheresis at BJH. Product is shipped to Penn.
4. The product is manufactured at CVPF. Cells are tested and cryopreserved.
5. Patients undergo further tests to include: H&P, brain MRI, CT C/A/P, PET-CT, EKG, Echocardiogram, PFTs, and labs.
6. Product is shipped to BJH cryo lab for storage.

Other Issues

- Supportive care is allowed. Antibiotics, growth factors, transfusions and other meds are permitted.
- Dose modifications or reductions are not allowed.
- CTCAE 3.0 will be used to assess toxicity.
- DLT is defined
  - Any grade 3 or higher non-hematologic toxicity
  - Any grade 3 or higher allergic toxicity
  - Any grade 3 or higher autoimmune toxicity that involves vital organs such as heart, kidneys, brain, eye, liver, colon, or adrenal
- Stopping rules
  - If any death occurs that maybe related to the investigational product
  - If two or more DLTs occur pending discussion with the IRB, FDA and regulatory sponsor and DSMC.
- DSMC will meet every 6 months.

Objectives

Primary

To determine the safety and tolerability of a split, fixed dose of autologous T cells transduced with lentiviral vector encoding an enhanced TCR after non-myeloablative chemotherapy.

Secondary

To determine the clinical activity TCR gene therapy as assessed by RECIST criteria and progression-free survival.

To determine the persistence of modified T cells in the peripheral blood and at tumor sites.

To determine the functional properties and phenotype of modified T cells from peripheral blood and tumor sites.

Secondary Objectives: Immune Monitoring

Hypothesis: Persistence of multifunctional TCR transduced T cells will correlate with clinical response, progression free survival and overall survival.

Objective: To evaluate persistence, function and migration of adoptively transferred /TCR transduced T cells. These correlative studies may allow identification of surrogate end-points for future trials.

Beatriz Carreno, PhD
Michael Kalos, PhD

Immune Monitoring Approach

<u>Objective:</u> To evaluate persistence, function and trafficking of adoptively transferred (TCR transduced) T cells

Peripheral blood

Persistence -Tetramer and RT-PCR

Function - Cytotoxicity, polychromatic flow cytometry (phenotypic and cytokine profile), antigen/cytokine proliferative responses

Surgically resected metastasis

Trafficking – Immunohistochemistry, RT-PCR, and molecular characterization of tumor infiltrating lymphocytes

Immune Monitoring (12 weeks)

Blood: daily for 2 weeks, then weekly x10.

Serum: baseline and after T cells and daily x2w

Apheresis: week 4 and week 8

Tumor biopsy: for non-responders to assess infiltration and function of T cells

Immune Monitoring: tetramer persistence

Apheresis

UPenn for product manufacturing

T cell product

T cell product sample to characterize starting product

Patient

16cc / 3cc

T cell product infusion

30% product @ d5 → 16cc / 3cc

70% product @ d6 → 16cc / 3cc

d7-9, d12-16 – 16cc / 3cc

Week 3 – 16cc / 3cc

Week 4 – 2L Apheresis

Week 5-7 – 16cc / 3cc

Week 8 – 2L Apheresis

Week 9-12 – 16cc / 3cc

Beatriz Carreno

Previous Experience

T cells with NY-ESO-1 and MAGE specificities exist in cancer patients (Baumgaertner et al. Ex vivo detectable human CD8 T-cell responses to cancer-testis antigens. Cancer Research. 2006;66:1912).

Melanoma patients have been treated with NY-ES0-1^{SLLMWITQCFLPVFT} T cell clone (dose 5x10e9) with safety and anti-tumor effects demonstrated (Hunder et al. NEJM 2008).

T cells transduced with the same NY-ES0-1^{c259} that we will use, and followed with high dose IL-2 have been given to 17 patients with melanoma or sarcoma with safety and anti-tumor efficacy (NCT00670748). Patients were dosed using a single infusion strategy at mean 5x10e10 T cells (range 1.6 - 13x10e10).

Potential Risks

Cyclophosphamide
- mesna

T cell infusional toxicities
- split dose infusion
- no IL-2 chase

Lentiviral transduction
- insertional toxicity not reported with T cells
- patients monitored for RCL, secondary malignancies

Tumor distension / inflammation

Potential Risks - 2

TCR "on target" effects:

- **Preclinical screening of MAGE^{a3a} and NY-ESO1^{c259} against a panel of 9 non-transformed cells did not uncover reproducible reactivity to any normal cells.**
- **In clinical studies, no on-target toxicities were described with the NY-ESO^{c259} TCR T cells given to 17 patients with melanoma/sarcoma treated with doses that exceed the dose we propose (Robbins et al, 2010).**

TCR "off target" effects:

- **Off-target GVHD like syndrome due to mis-pairing possible (Bendel et al, Nat Med 2010).**
- **Clinically, this has not been observed in >143 patients treated with TCRs directed to a variety of TCR specificities (p53, CEA, NY-ESO-1, gp100, MART-1) (S. Rosenberg, OBA/RAC presentation 12/09).**

The doses used in the above trials exceeds the flat dose (1x10e10) that we propose.

Dose Rationale and Toxicity Management

The patients will be dosed with NY-ESO-1^{c259} (previous clinical experience) or with MAGE^{a3a} (no previous clinical experience) at 3x10e9 cells on day 5 and 7x10e9 cells on day 6.

Considering the distribution of cancer testis antigens, and previous extensive clinical experience with adoptively transferred T cells with engineered TCRs, we propose a flat dose of 1x10e10 cells. We believe that this is the NOAEL dose for adoptively transferred T cells with the available pre-clinical and clinical data set. Given the previous successful dosing of patients with NY-ESO-1^{c259} at up to 1.3x10e11 cells, our proposed dose is a ~1log10 safety reduction.

The recent toxicities of CARs are <u>not</u> relevant to TCR protocols, given the physiologic function of TCRs/CTLs.

In addition to dose reduction, protocol 1057 incorporates two additional features that are expected to reduce the potential for toxicity: 1) split dose infusions and 2) omission of high dose IL-2.

Questions?

Thank you for your time and effort