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

Protocol #1007-1056

A Phase I, dual cohort, two-site, clinical trial evaluating the safety and activity of redirected autologous T cells expressing a high affinity TCR specific for MAGE-A3/6 or NYESO1 administered post ASCT in patients with advanced myeloma

September 17, 2010

Penn Medicine
Abramson Cancer Center

Adaptimmune
Transforming T Cell Therapy

Protocol 1056: Investigative Team

- **STUDY CHAIR:**
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- **Sponsor:**
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  Abramson Cancer Center, University of Pennsylvania

- **Financial Sponsor and Collaborator:**
  Bent Jakobsen, Ph.D.
  Adaptimmune Ltd.

Protocol 1056: Investigative Team

Clinical Trial Site - University of Maryland:

Principal Investigator:	Aaron P. Rapoport, MD	Protocol Chair
Sub-Investigators:		
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	Ming Tan, Ph.D.	

Clinical Trial Site - University of Pennsylvania (Coordinating Center):

		Role
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Sub- Investigators:	David Porter, MD	Clinical
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	Michael Kalos, PhD	Correlative Analysis
	Ravi Amaravadi, MD	Medical Monitor

Overall Sponsor/Sponsor of Cell Manufacturing (BB-IND PENDING)
University of Pennsylvania:
Carl H. June, M.D., Director of the Translational Research Program
Bruce L. Levine, Ph.D., Director of the Clinical Cell and Vaccine Production Facility

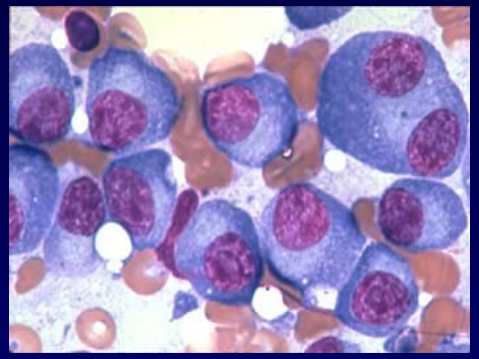
Financial Sponsor
Adaptimmune Ltd.
Bent Jacobsen, PhD-Chief Scientific Officer
Helen Tayton-Martin, PhD MBA-Chief Operating Officer
James Noble-Chief Executive Officer

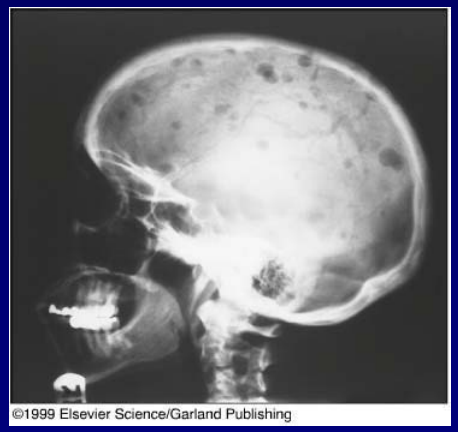
Outline

- Myeloma background
- Design and testing of affinity enhanced TCRs
- Pre-clinical testing
- Protocol design and safety features to minimize toxicity

Multiple Myeloma

- Approximately 20,580 new cases and 10,580 deaths from MM are expected in the United States in 2009
- Slightly more common in men than in women
- Incidence in blacks is approximately twice than that in whites
- Mean age at diagnosis is 62 yrs for men and 61 yrs for women

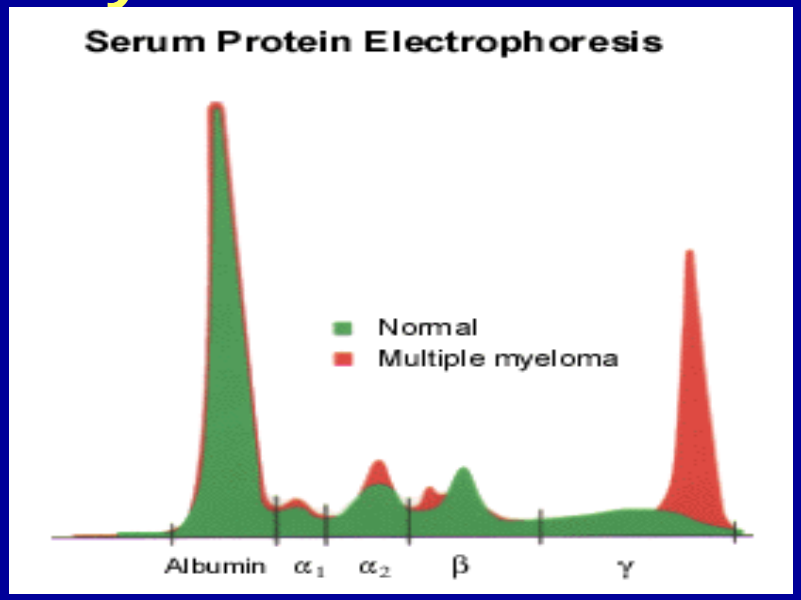




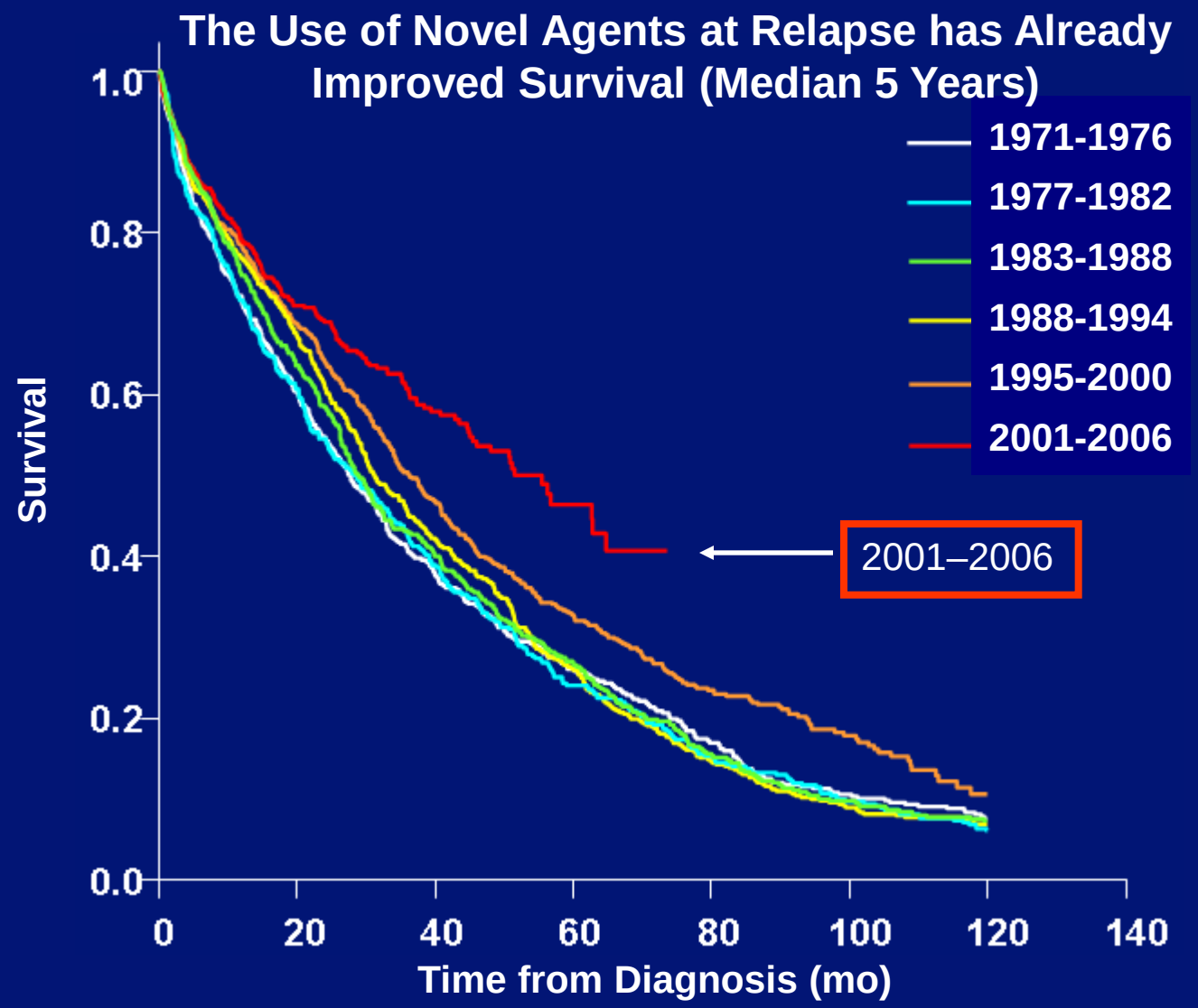
Cancer facts and figures 2009. American Cancer Society; 2009. Horner MJ, et al, eds. SEER cancer statistics review, 1975-2006. National Cancer Institute. NCCN Practice Guidelines. V.3.2010.

Multiple Myeloma

- Clinical Features
  - Presence of monoclonal protein
  - Bone destruction (lytic bone lesions)
  - Anemia
  - Renal failure
  - Increased risk of infection
  - Hypercalcemia
  - Neurologic
    - Spinal Cord Compression
    - Peripheral Neuropathy
    - Hyperviscosity



Overall Survival is Improving



Kumar SK et al. *Blood*. 2008;111:2516

Multiple Myeloma

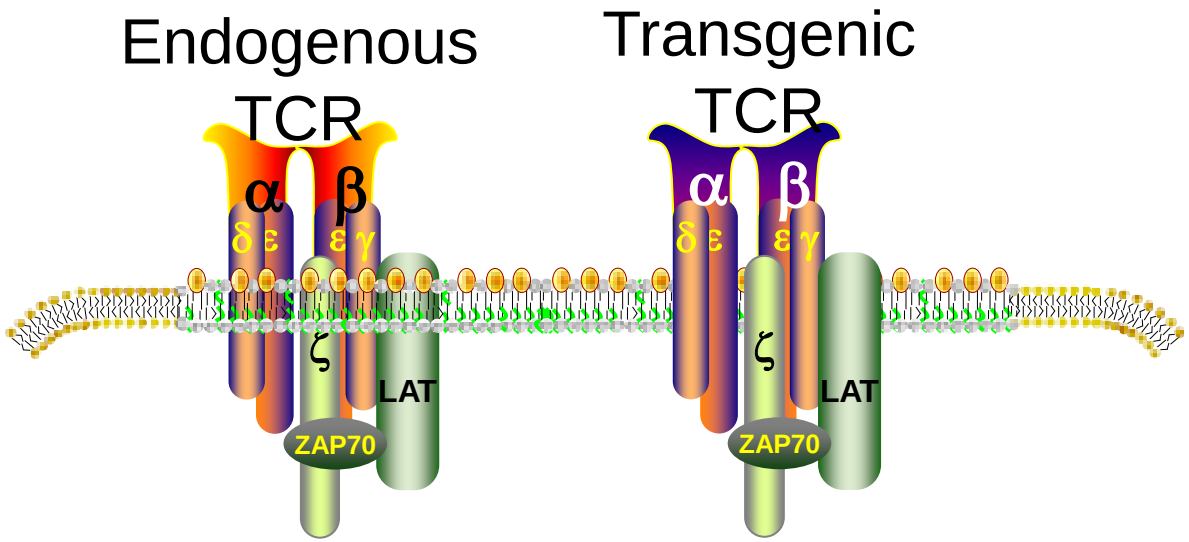
- Currently incurable in most patients
- Standard chemotherapy + targeted therapy (thalidomide, lenalidomide, bortezomib) response rates: 50% to 70%
  - Long-term complete responses: rare
  - Median survival with standard therapy: 3 yrs
- Stem cell transplantation prolongs survival, but not curative
- Treatment of relapse
  - No standard second line therapy
  - Unmet medical need

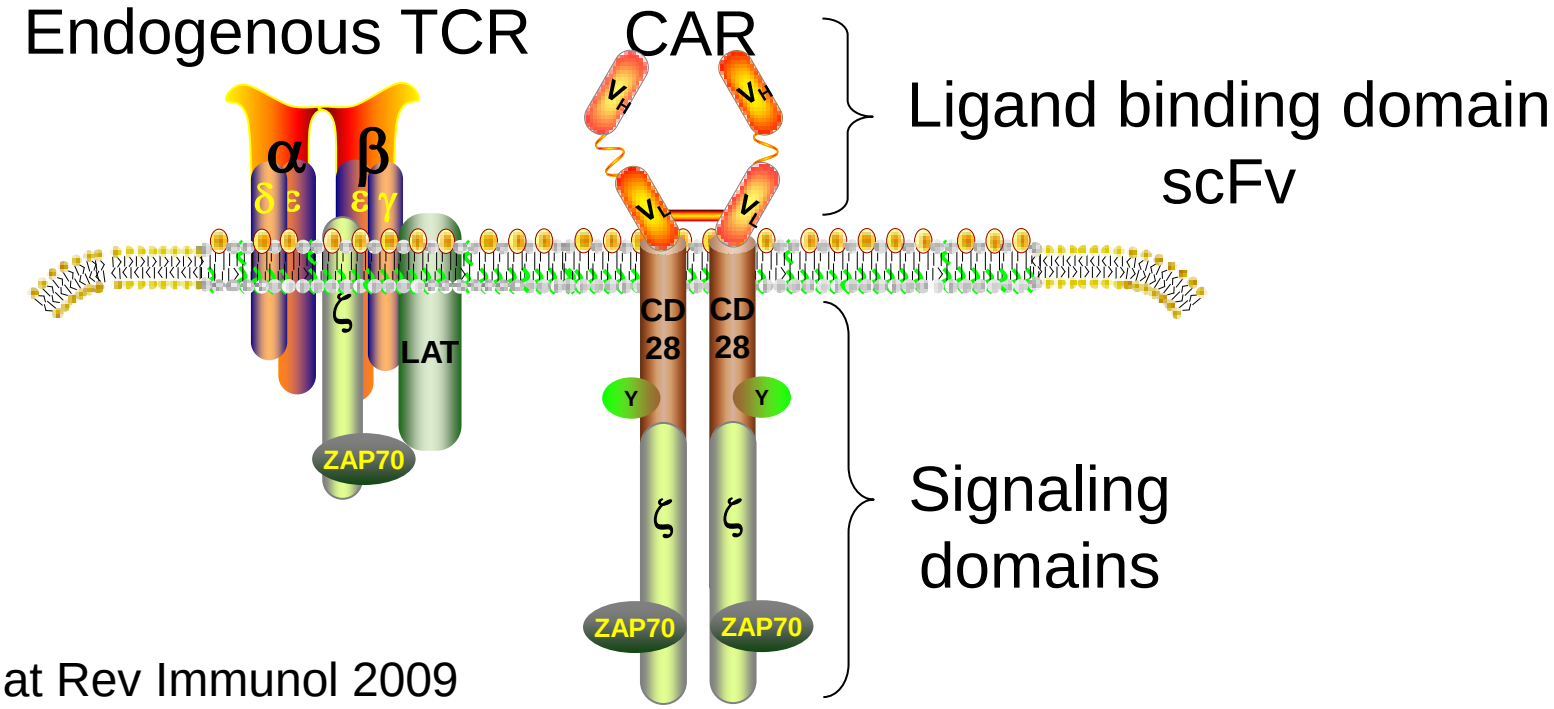
Greipp et al Journal of Clinical Oncology. 2005; Porrata et al, Blood 2001; Pavletic et al, BMT 1998; Roux et al, Blood 1996; Hakim et al, Blood 1997

Myeloma: immunology aspects

- Retrospective studies suggest better clinical outcomes after auto transplants with more rapid lymphocyte recovery [Porrata et al].
- Myeloma-reactive T-cells present at low frequencies in the marrow or blood of myeloma patients [Dhodapkar, et al].
- Allogeneic BMT: long term disease control suggests potent allo immune effects, but high transplant-related morbidity and mortality [Badros et al].

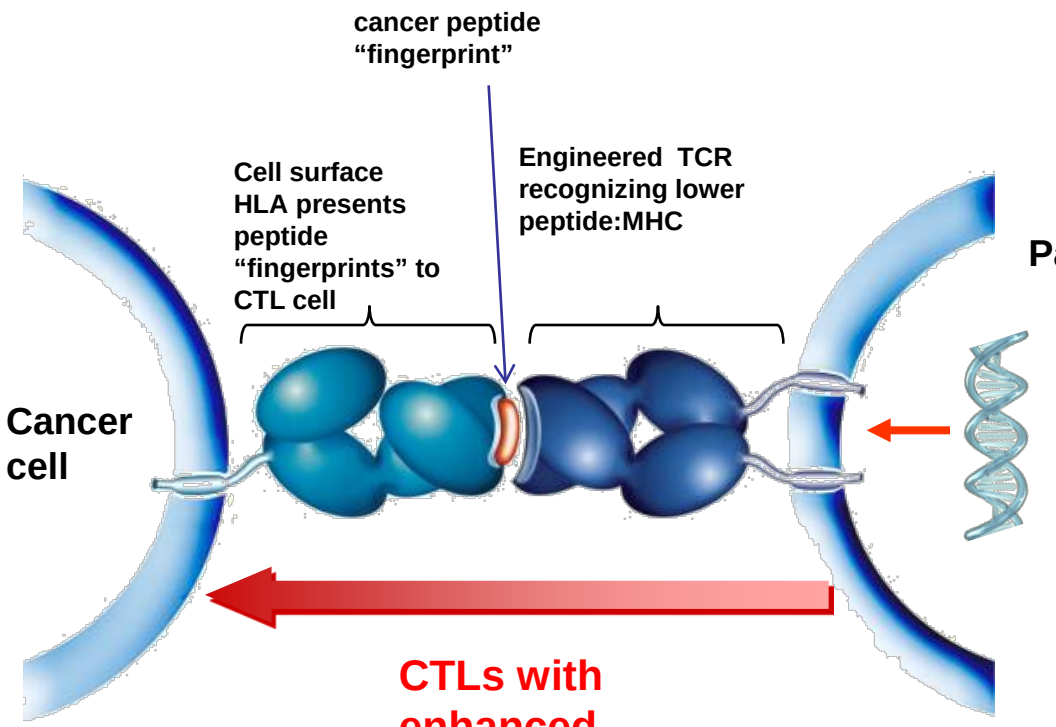
Therapy with genetically retargeted T cells





June et al, Nat Rev Immunol 2009

Rationale for therapy with natural and genetically retargeted CTLs



Patient’s natural CTL

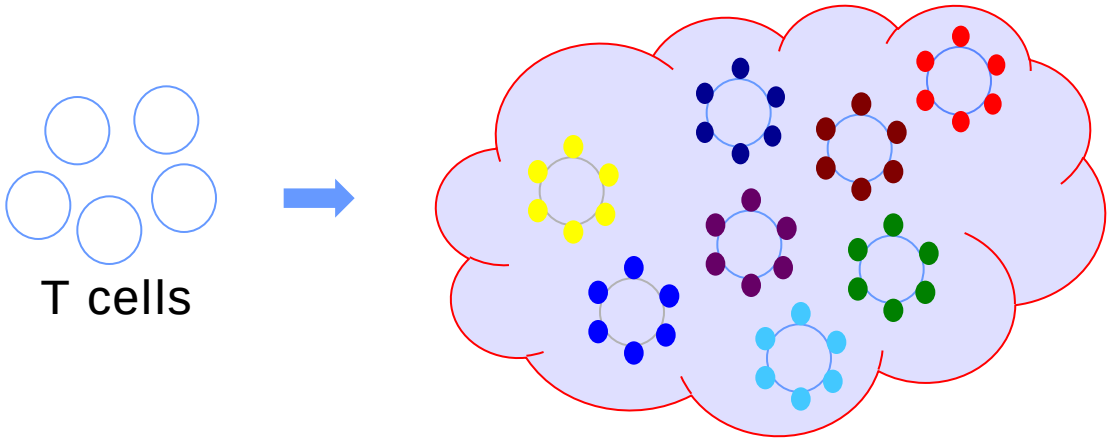
DNA encoding the new engineered version of the killer T cell receptor is given to the patient’s own isolated killer T cells

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

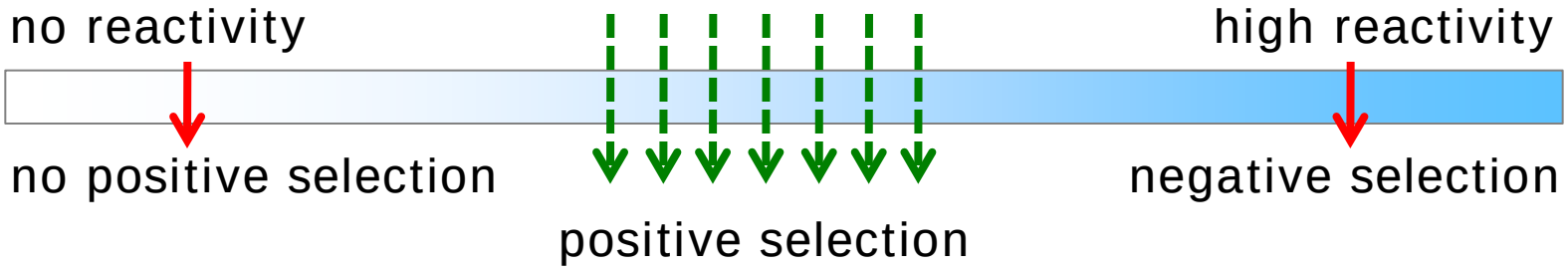
Rationale for therapy with natural and genetically retargeted CTLs

- **HLA-presented peptide antigens are the largest class of cancer-specific targets**
  - HLA class I peptide antigens provide cell surface ‘flags’ for expression of intracellular antigens
  - Biological function of class I antigens understood
  - Many antigens have essential functions
- **Cytotoxic T cells (CTLs) have vastly more cytotoxic potential than any single-mechanism drug or biologic agent**

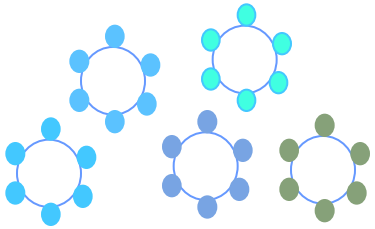
TCR triggering thresholds are restrained by selection in the thymus



TCR expression and selection in the thymus



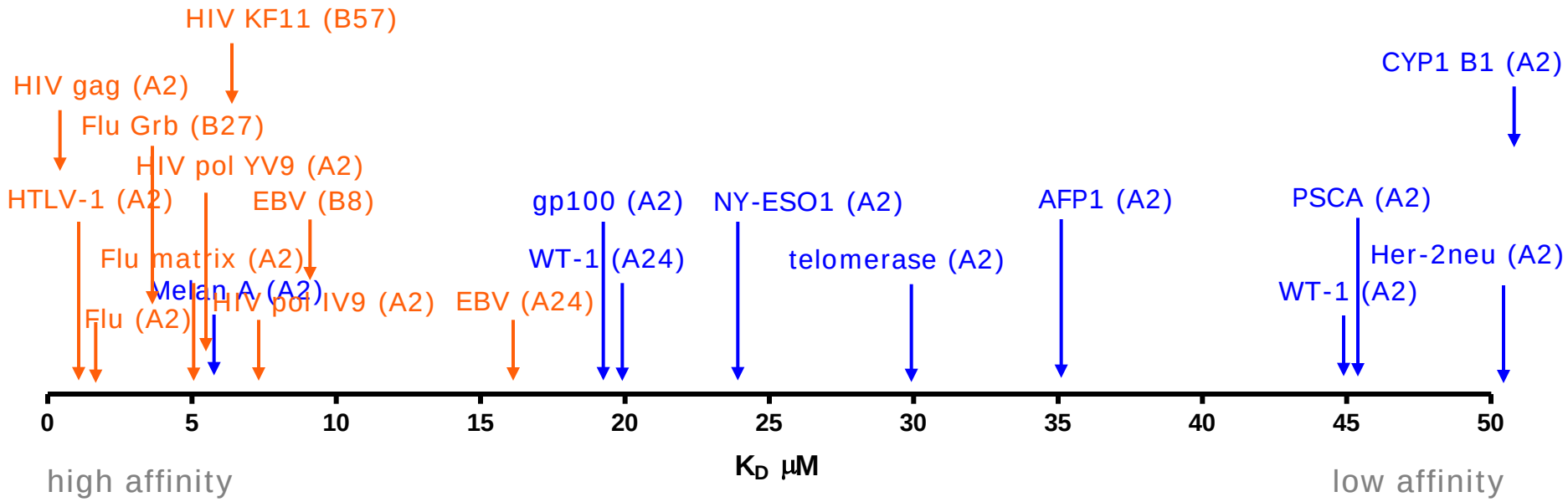
circulating T cells
~ 25 x 10^6 specificities



Good viral responses
Poor cancer responses

Savage and Davis, Immunity 2001

TCR affinities for cancer versus viral antigens



Blue = cancer antigen specific TCRs

Orange = viral antigen specific TCRs

Immunocore Unpublished Data

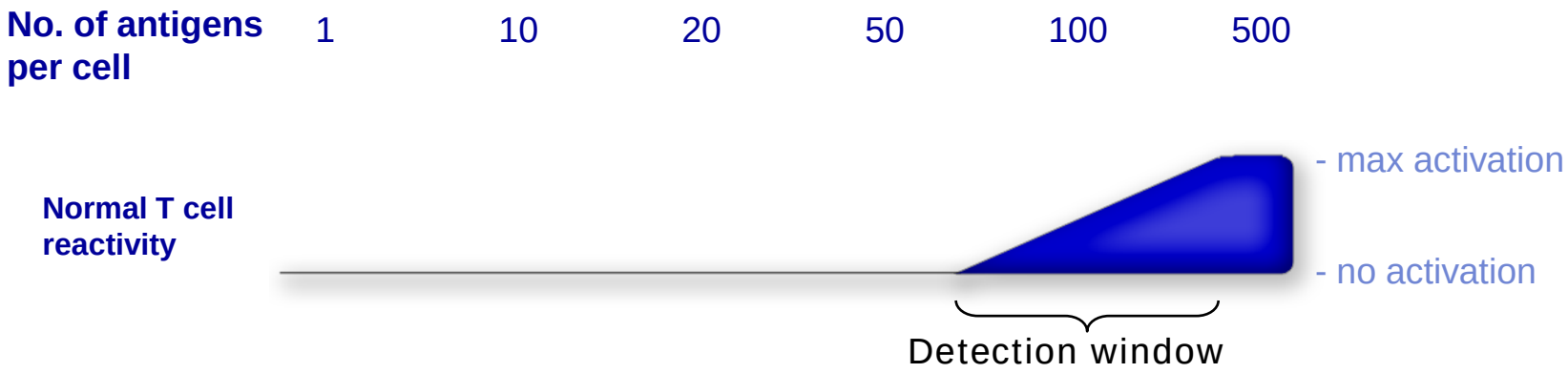
Concept supported in Cole et al. J Immunol, 2007; Stone et al. Immunology 2009

T cells with natural TCRs for cancer antigens are usually tolerant (non-reactive) to tumors

- TCRs for cancer antigens are low affinity, unlike viral TCRs
- Cancer cells often have low level expression of HLA class I (Vitale et al, Clin Cancer Res 2005)
- Individual melanoma and myeloma cells present an average of only 10-50 copies of the NY-ESO-1$^{(157-165)}$/HLA-A*0201 epitopes per cell (Purbhoo et al. J Immunol, 2006)

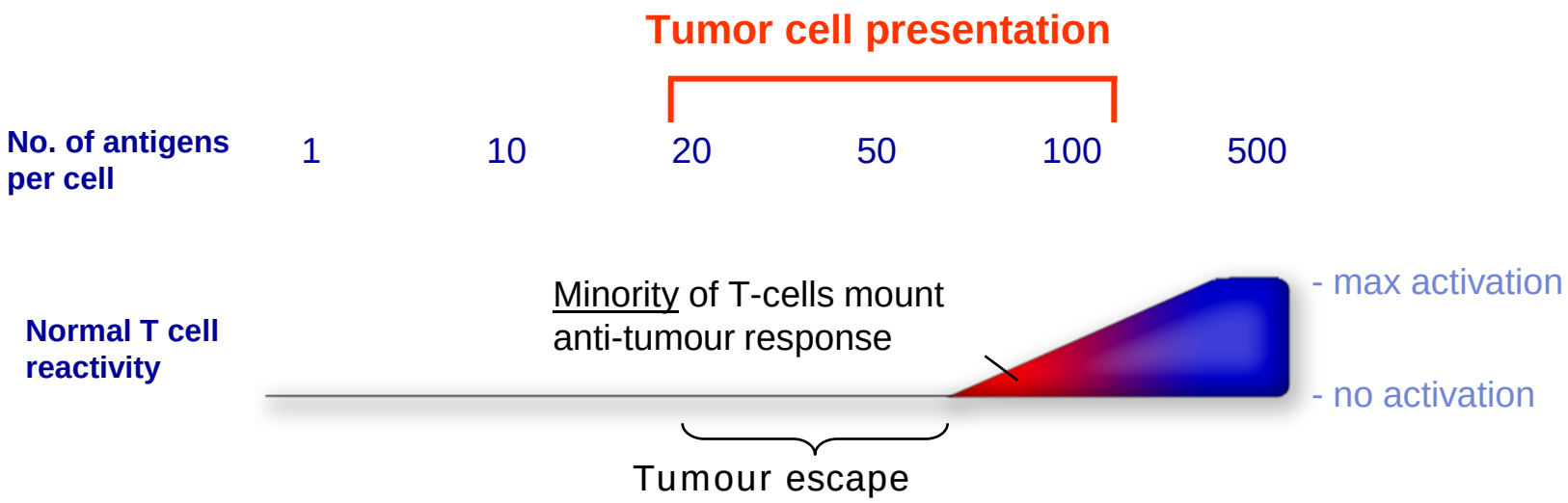
<u>Terms</u>
- Affinity
- Avidity
- Recognition efficiency
- Epitope
- Antigen

The selection of TCRs in the thymus blunts potential anti-tumor immunity



Positive selection during maturation restricts natural CTLs to a narrow epitope detection window

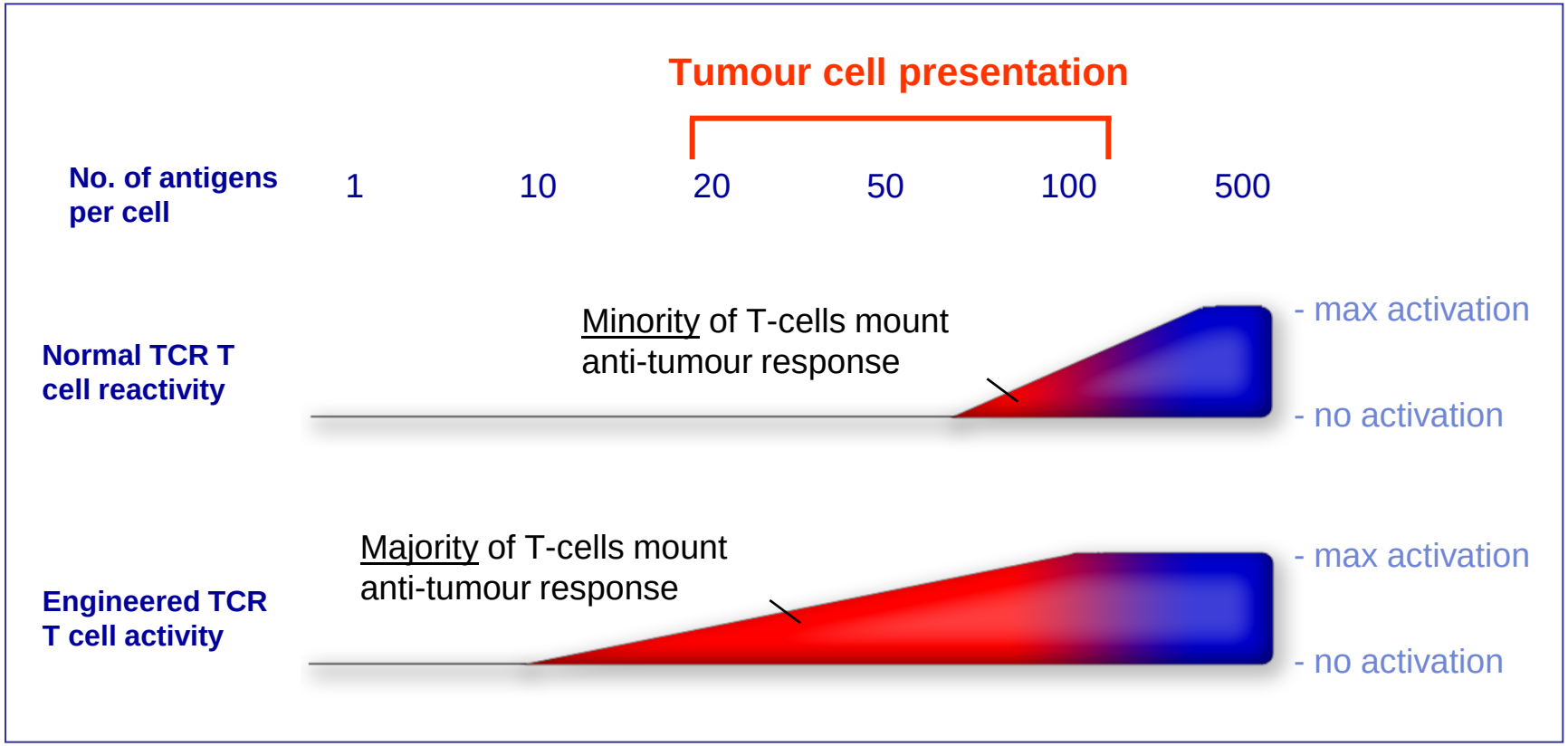
Cancer cells often present too few antigens to activate conventional CTLs



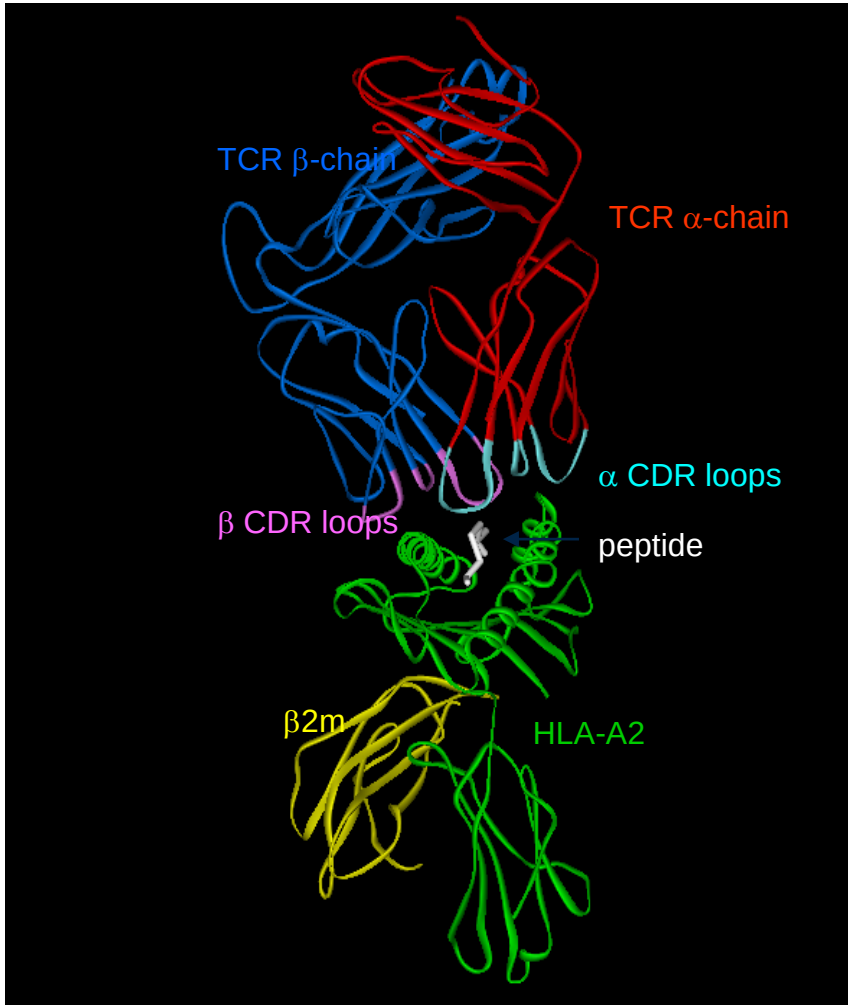
Corollary: "Tumor escape window" may explain why cancer vaccines to non-viral induced tumors often fail

Immunocore Unpublished Data
Concept supported in: Stone et al, Immunology 2009; Schmid et al. J Immunol 2010

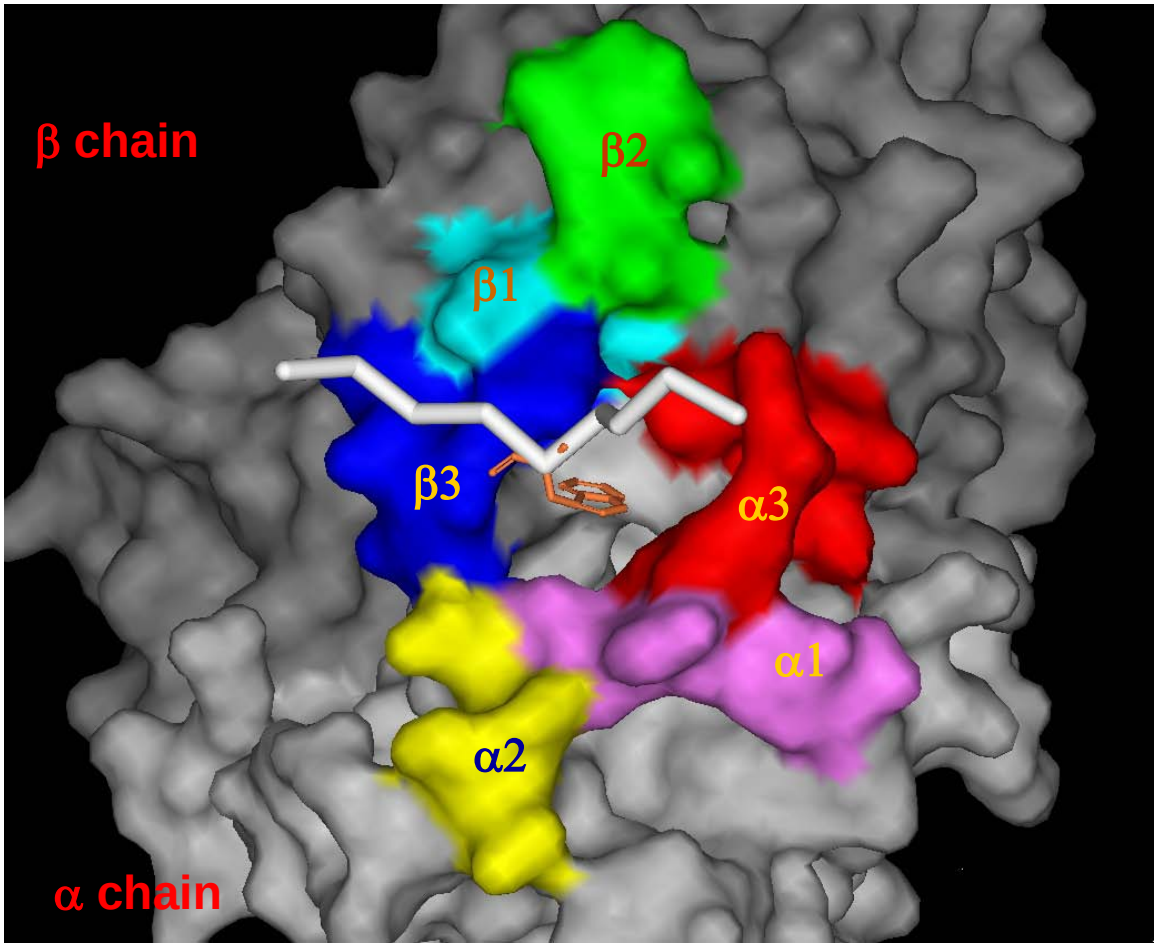
Potential of enhanced affinity TCRs to overcome tumor escape



TCRs bind MHC-peptide through CDR loops



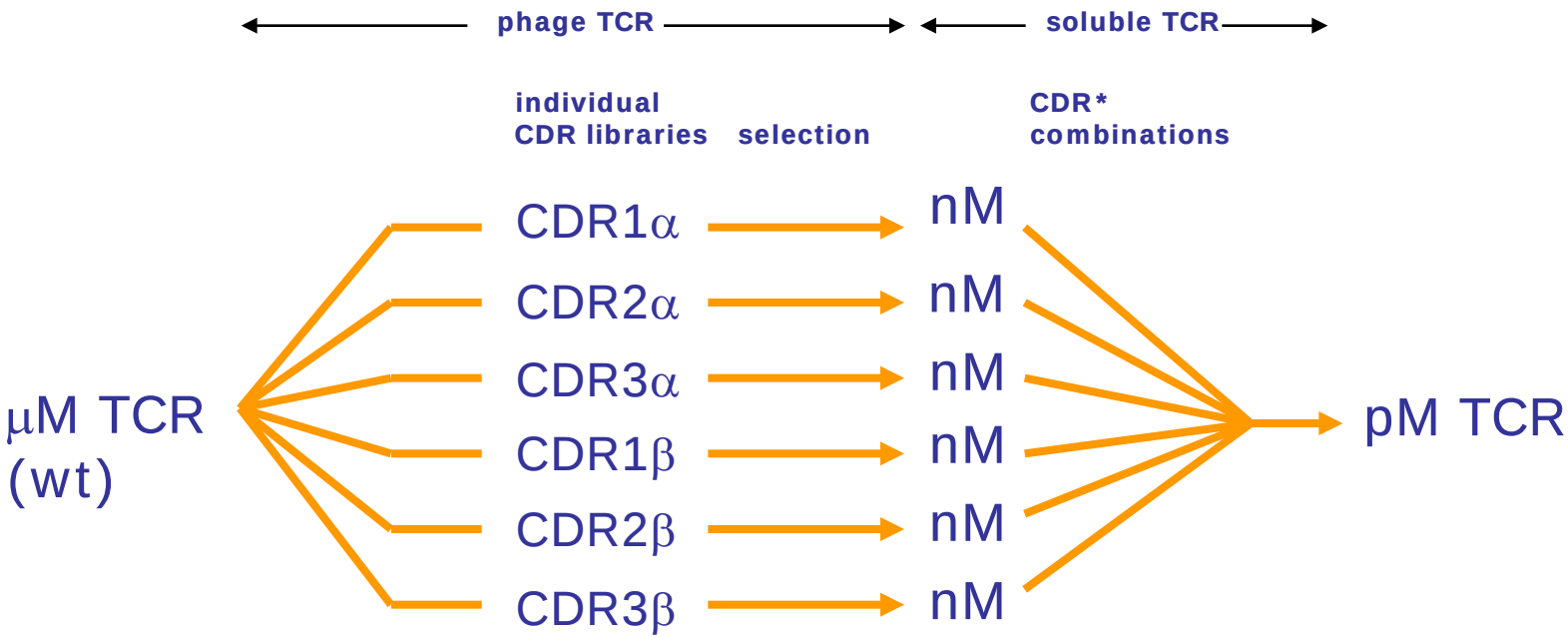
Main contacts to peptide are made by CDR3s



CDR2s are primary contact points to MHC

Molecular evolution to optimize TCR affinity

2 step affinity generation: phage display selection and CDR* combination



Boulter et al. **Protein Engineering** vol. 16, no. 9 pp. 707-711, 2003
Yi et al. **Nature Biotechnology** vol. 23, no. 3 pp. 349-354, 2005
Varela-Rohena et al. **Nature Med**. Vol. 14, pp.1390-1395, 2008

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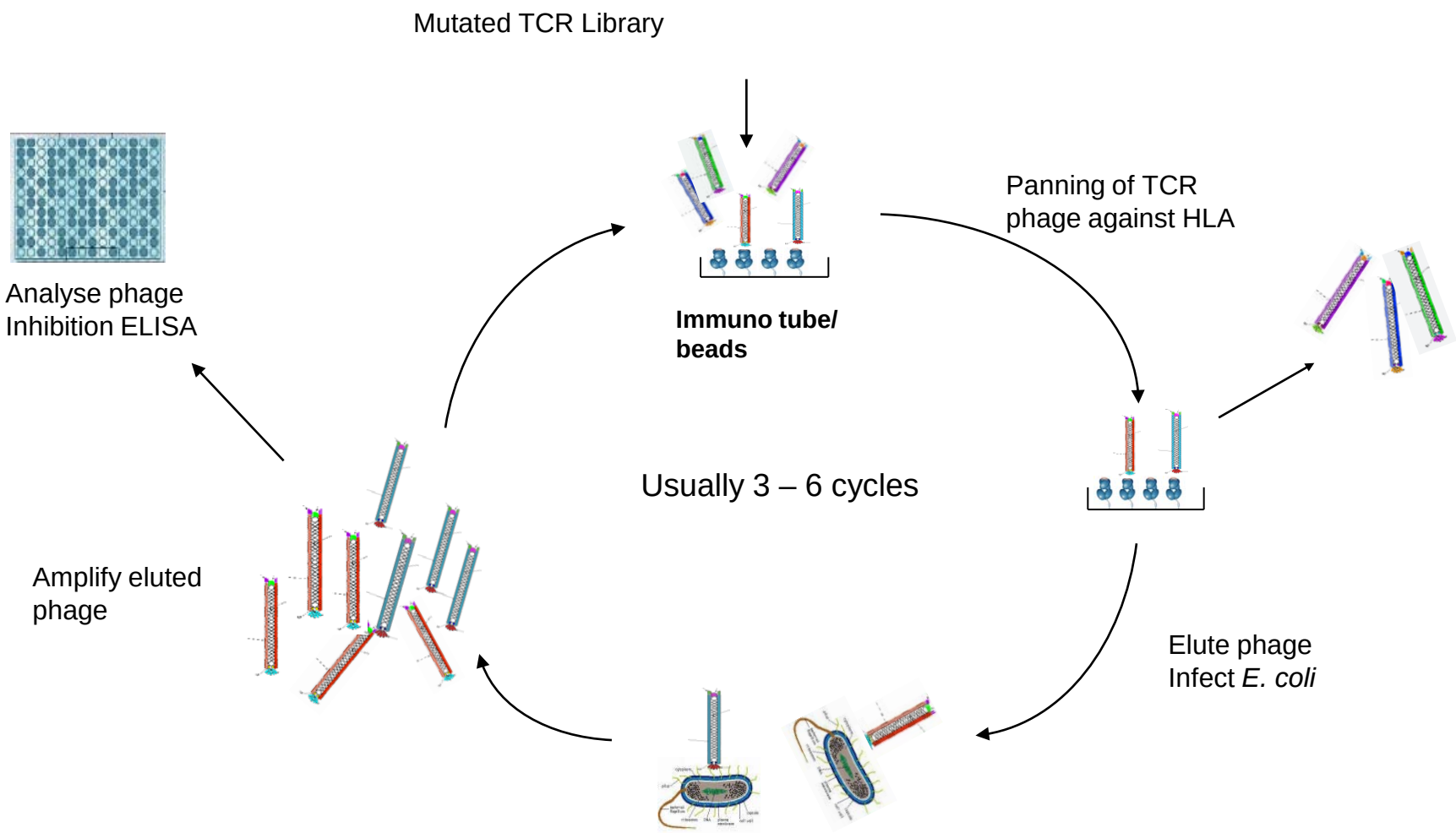
Generation of affinity optimized TCRs targeting NY-ESO-1 and MAGE A3/6 – selection on phage



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Rationale to target cancer testis antigens

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

	MAGE-A3	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/

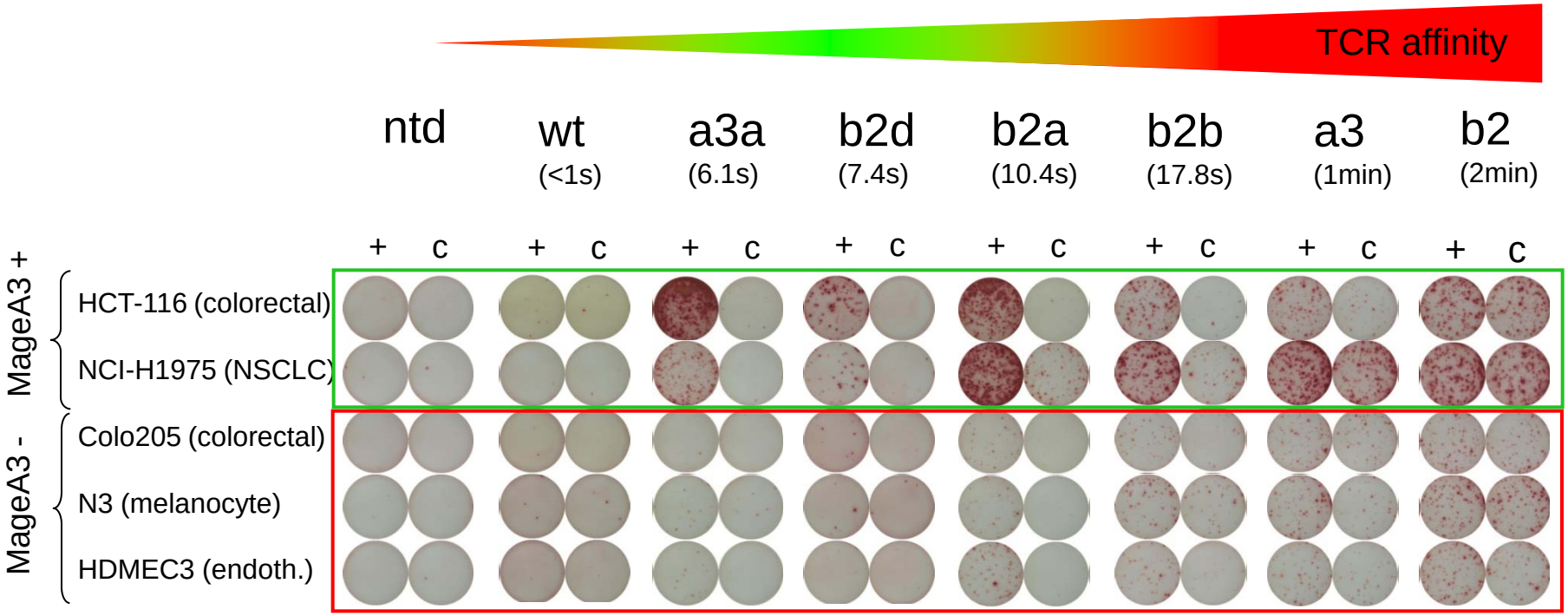
Generation of affinity optimized TCR targeting MAGE A3/6 (HLA A1)

TCR	wt*	a3c	a3a	b2d	b2a	b2b	a2	a3	b2	b1	a3b3 #
$T_{1/2}$	-	<1s	6.1s	7.4s	10.4s	17.8s	46s	66s	2.0min	4.4min	6.5hrs
Kd	300uM	55uM	6.55uM	6.7uM	9.4uM	6.3uM	62nM	190nM	169nM	61nM	58pM

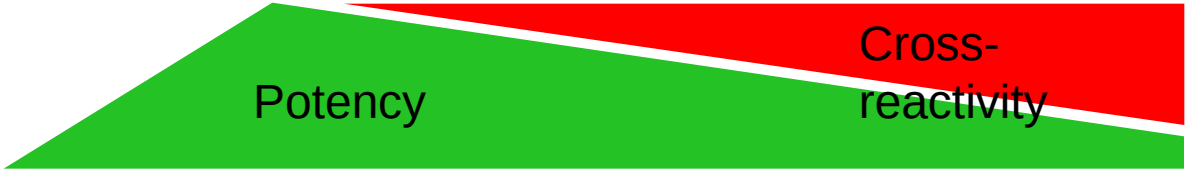
* wtTCR: kinetics of binding too fast to determine accurately (T1/2 <<< 1s)
\# a3b3 TCR: highest affinity mutant – blocking reagent

- 10 TCRs (1 parental and 9 affinity enhanced) evaluated
- Epitope: HLA-A*0101 MAGE A3$^{(168-176)}$-EVDPIGHLY (Celis et al, 1994)
- Preclinical studies focused on the fundamental question of specificity vs. potency
- TCR a3a selected for clinical trial. This TCR has 4 α-chain mutations and a wild type β-chain.

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



Blocking sTCR (a3b3, t½ 6.5 hours)



+ = Target cells & TCR transduced T cells

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

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Functional assays to identify optimized TCR T cells
Experimental Design

Candidate TCRs

T cells → CD3/28 bead activation → LV transduction TCR MAGEA3: wt, a3a → Restimulation/expansion KT64, 86, 41BBL+OKT3 aAPC →

Degranulation assays
Cytokine secretion
Cytolytic activity
Humanized mouse tumor model

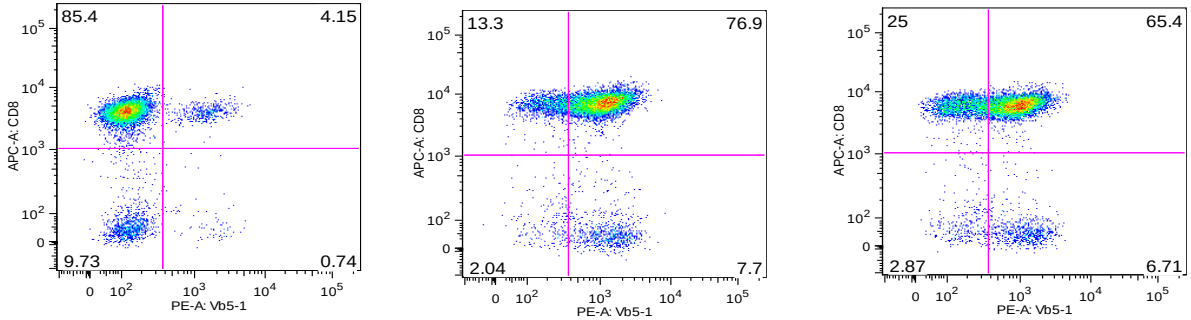
Affinity enhanced MAGE-A3 TCR mediates robust degranulation against MAGE-A3/HLA-A*01-positive targets

Un-transduced **WT** **a3a**

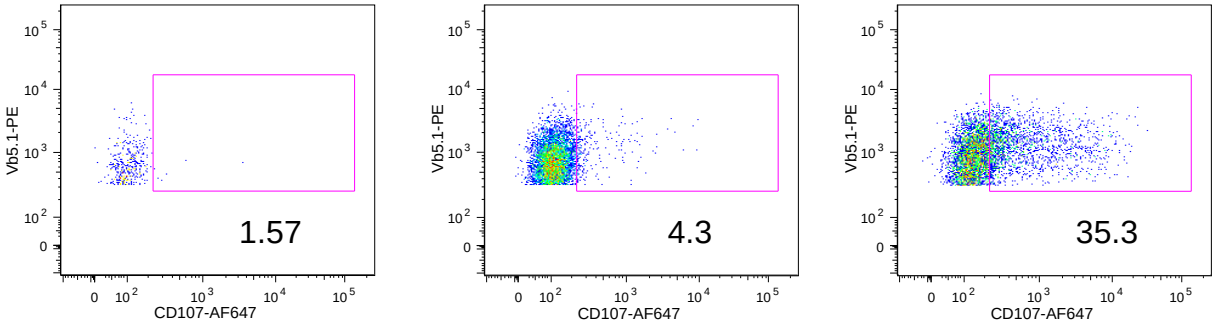
Transduction efficiency

CD8

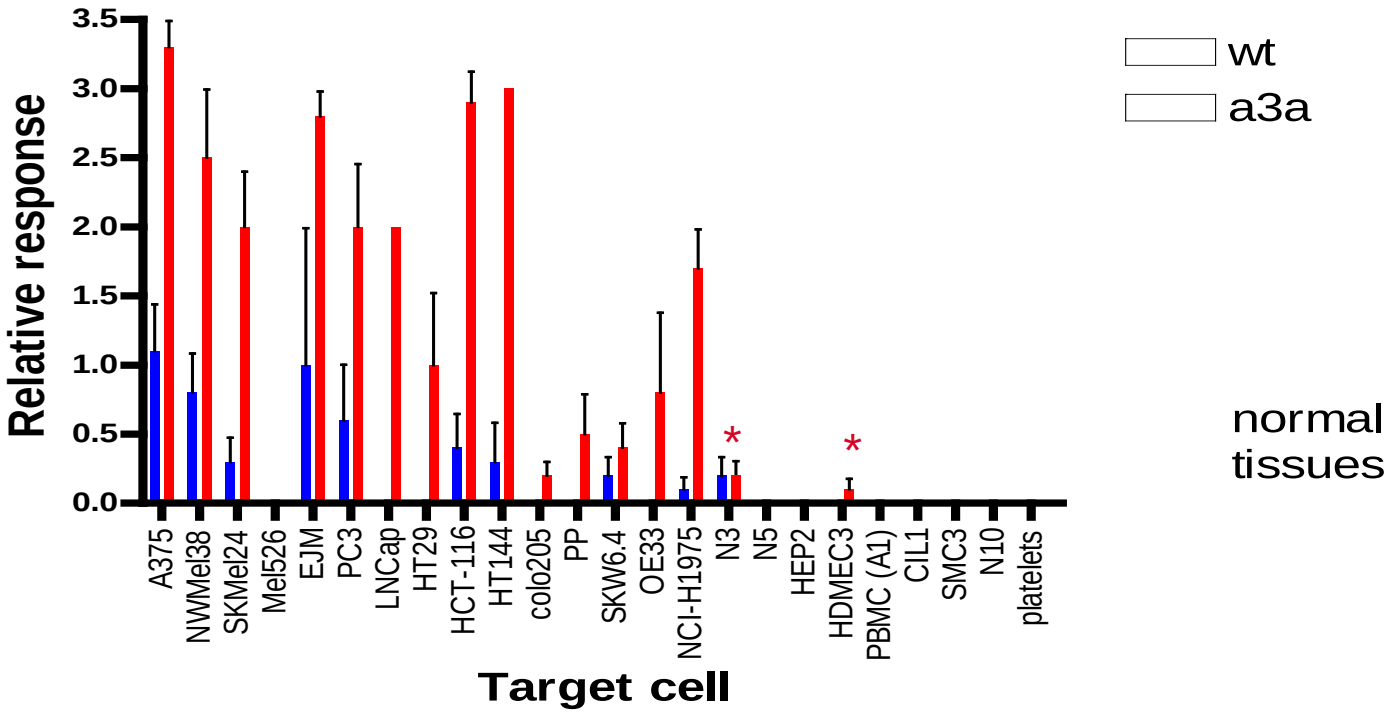
Vβ5.1



OV79 (HLA-A*1+, MAGE-A3+)



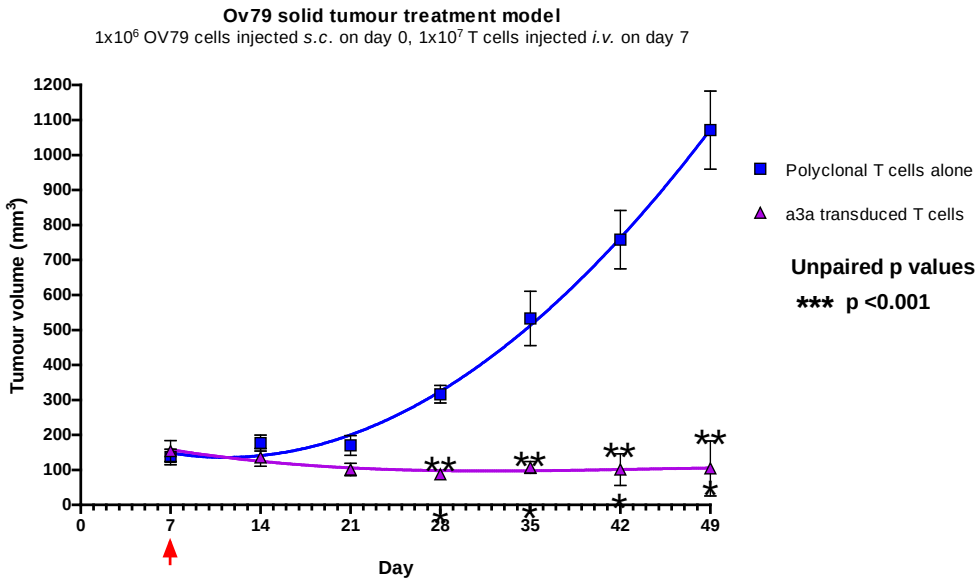
IFN-γ response of MAGE-A3 TCR transduced T cells to various tumor and normal cell lines



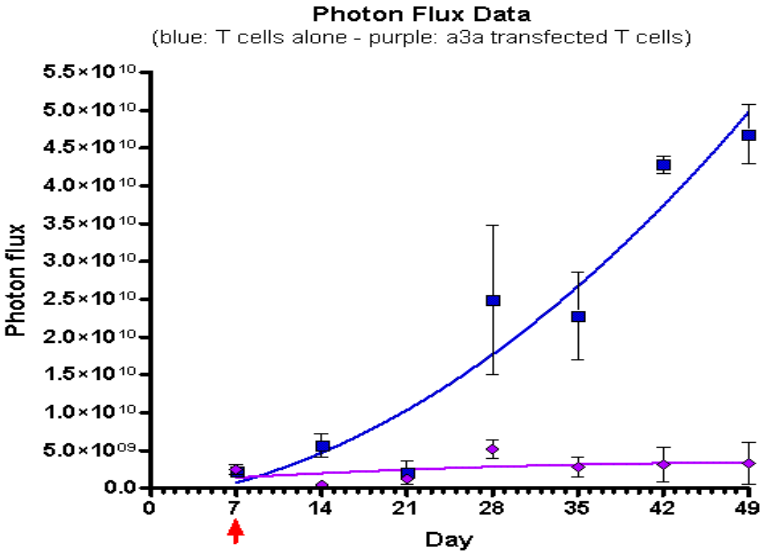
*N3 cells: response shown above only present in 3/8 assays
HDMEC3 cells: response shown above only present in 1/7 assays

MAGE-A3 TcR-transduced T cells mediate potent activity in an established solid tumor model

A. Tumor volume (mm^3)



B. Photon flux



3 of 5 animals tumor free out to day >100
1 animal tumor stable

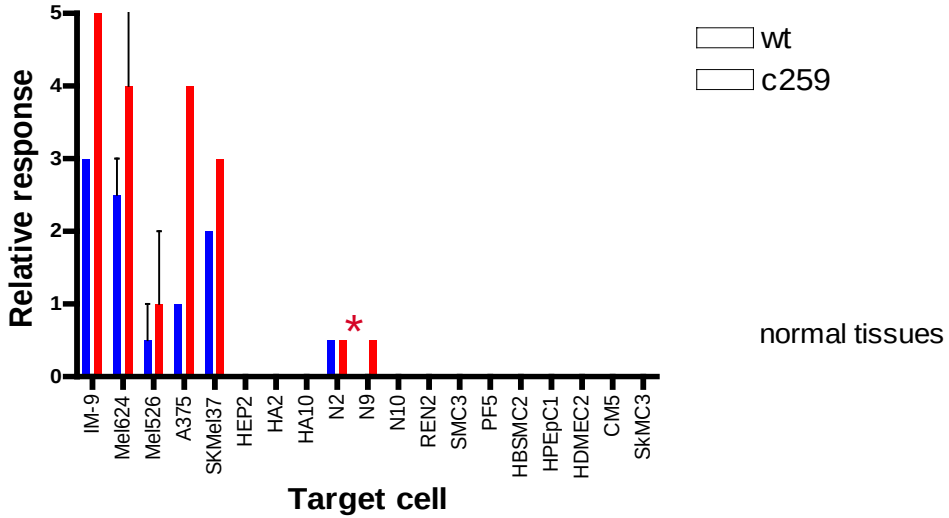
❖ ***anti- MAGE-A3 high affinity TCR mediates functional activity against established MAGE-A3/HLA-A*01-positive solid tumors***

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
  - 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-A3a3a TCR but no off target effects seen in patients
N9: results shown in 3/3 experiments no activity in LDH killing assay

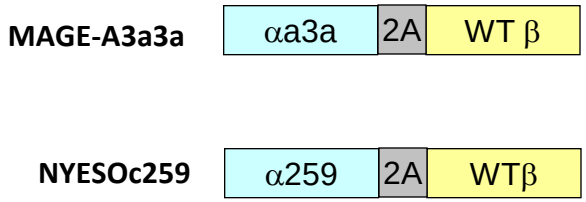
Protocol 1056

A Phase I/II, dual-cohort, two-site, clinical trial evaluating the safety and activity of redirected autologous T cells expressing a high affinity TCR specific for MAGE-A3/6 or NY-ESO-1 administered post ASCT in patients with advanced myeloma

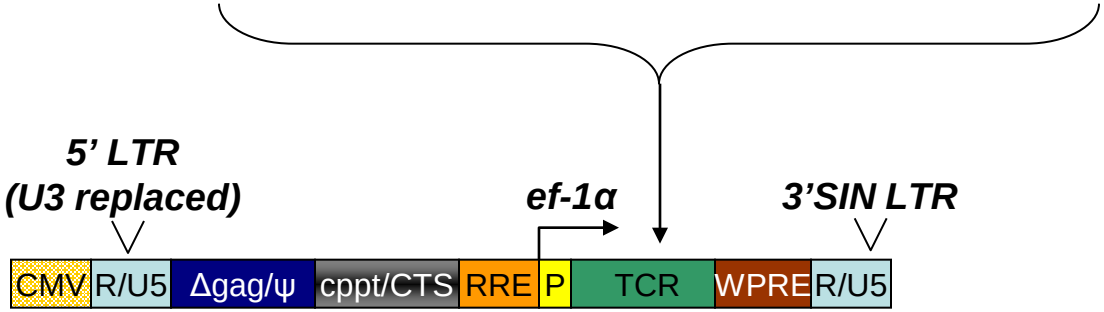
Protocol 1056: manufacturing overview

GMP vectors manufactured at City of Hope

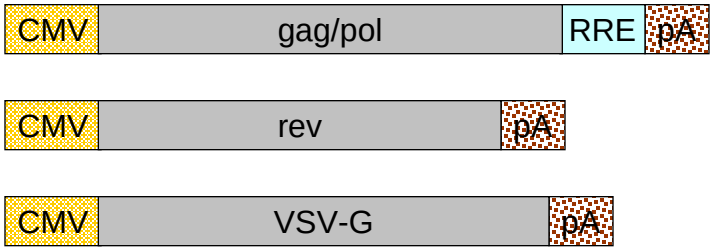
Transgenes



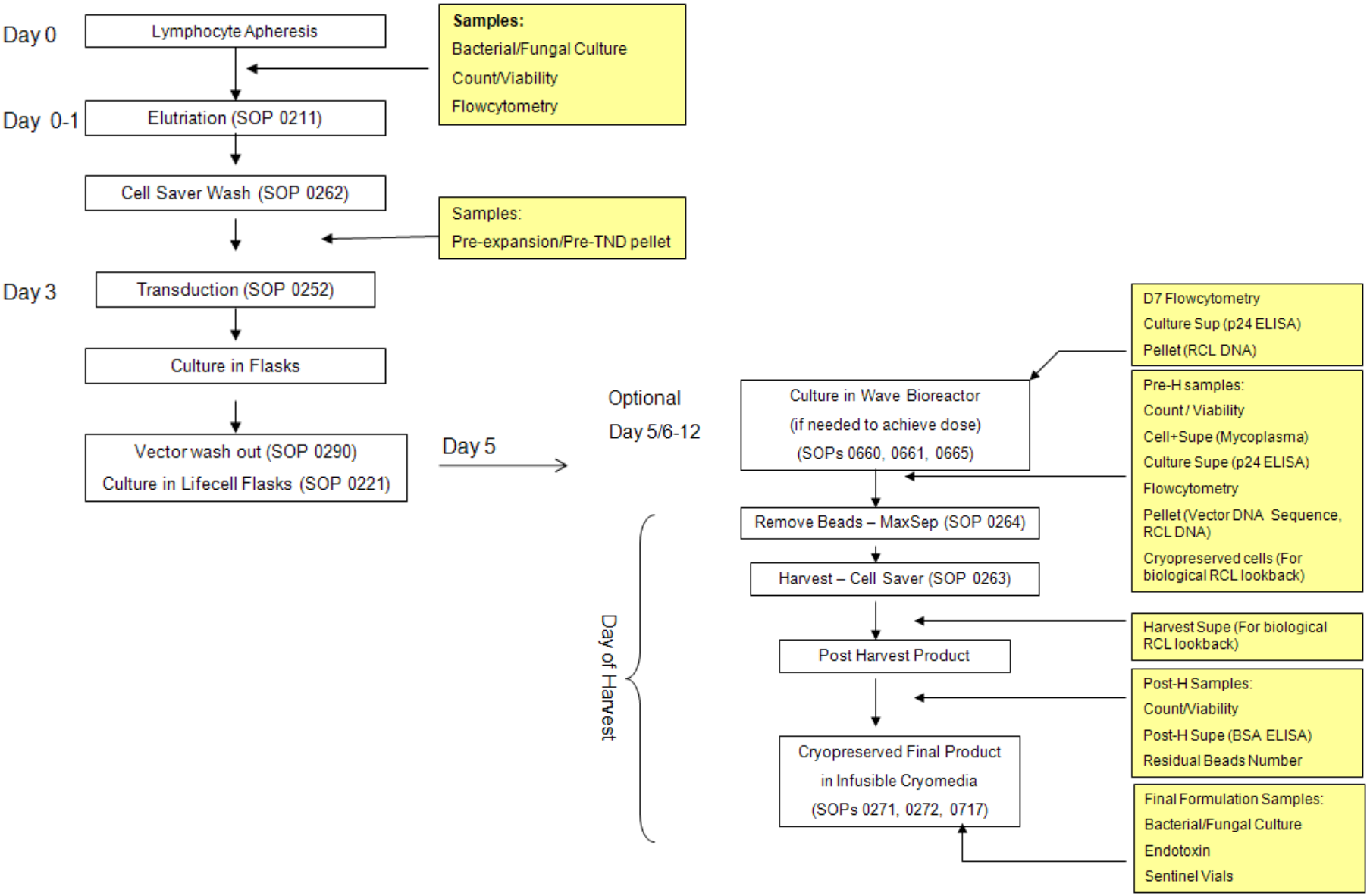
Vector



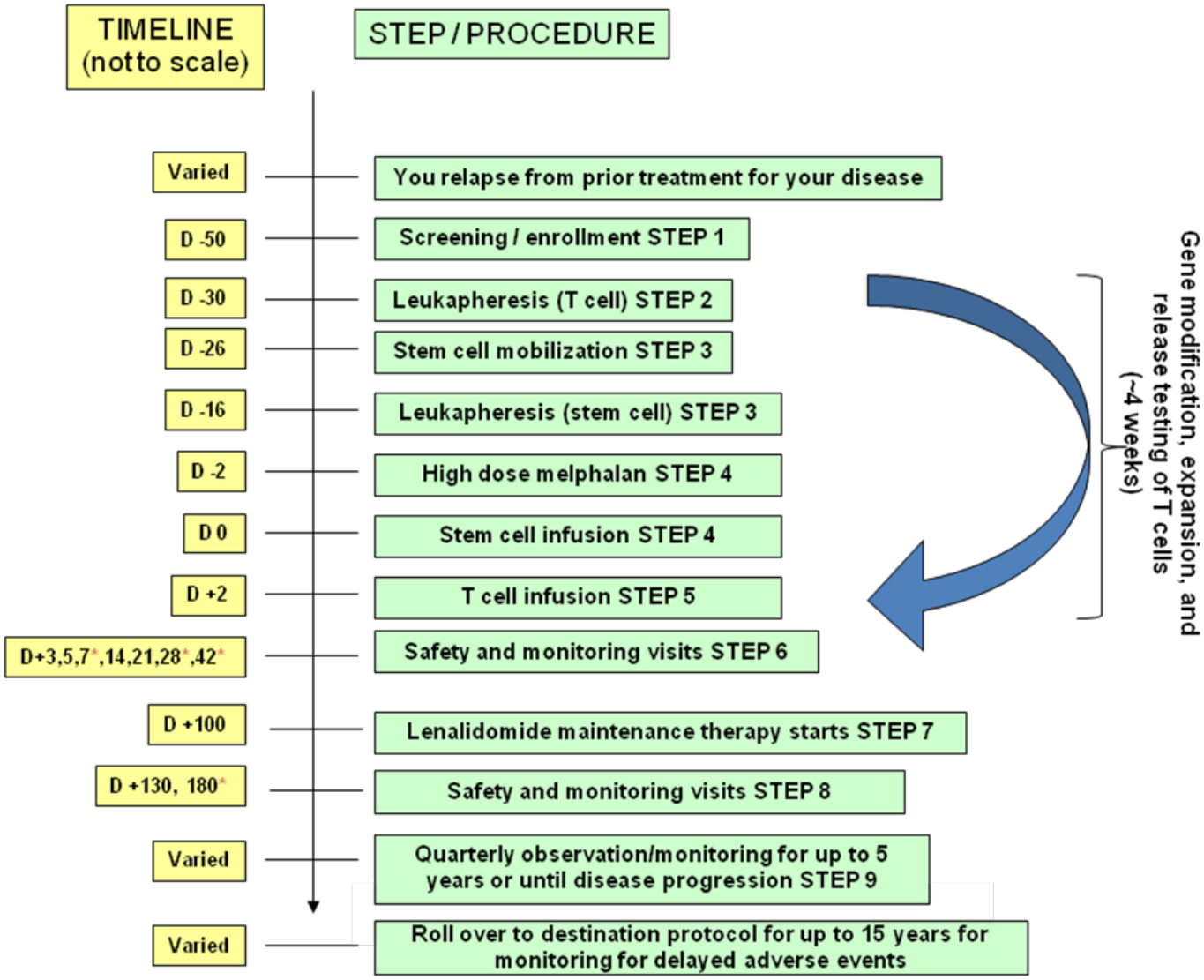
Packaging Plasmids



Cell manufacturing process: CVPF at UPenn



Study Design



** Bone marrow biopsy at these timepoints*

Primary Objectives

- To evaluate the safety and tolerability of autologous genetically modified T cells transduced to express affinity optimized MAGE-A3 TCR in HLA-A1 subjects, and affinity optimized NY-ESO-1 TCR in HLA-A2 subjects with advanced myeloma.

- To measure the incidence of autologous GVHD in patients following infusion of TCR modified autologous T cells.

Study Cohorts and Dosing Considerations

- Cohort 1:

  -N=6

  -HLA-A2 and NY-ESO-1 antigen positive by RT-PCR
- Cohort 2:

  -N=6

  -HLA-A1 and MAGE-A3 or MAGE-A6 antigen positive by RT-PCR or IHC
- Genetic randomization. Staggered infusions
- Flat dose: 1e10 T cells with no IL-2 infusion
- Schedule: day 2 post stem cell infusion

Dosing rationale: considerations

- Previous trials with autologous T cell transfer therapy in myeloma patients in the setting of HSCT
- Previous trials with lentiviral engineered T cells
- Rationale for omitting IL-2 infusions post T cell dosing
- Expected safety profile of engineered TCRs and CARs
- Previous TCR trials with CTLs

Previous trials with autologous T cell transfer therapy in multiple myeloma patients in the setting of HSCT

- **Trial #1: CD3/28 stimulated adoptive T cell transfer leads to rapid lymphocyte recovery (Rapoport et al, Nat Med, 2005).**
  - Randomized trial design, 54 patients enrolled; 52 patients infused.
  - Single infusion. Mean Dose 8.02 x 10e9 CD3+ cells (range, 1.6 – 11.1) on day +12. No IL-2. No SAE.
- **Trial #2. Day +2 infusions lead to lymphocytosis and engraftment syndrome in a subset of patients (Rapoport et al, Clin Cancer Res, 2009).**
  - Randomized trial design, 50 patients. Dose 4.26e10 (range, 1.59-5.0) T cells day +2. No IL-2.
  - Schedule dependent effects of day +2 vs day +12 infusions post melphalan.
  - Homeostatic expansion of infused T cells, with T cell leukocytosis.
  - Immune reconstitution: T reg depletion. Elevated endogenous IL-15.
  - Early T cell infusions are associated with a prolonged leukocytosis and an engraftment syndrome. No dose limiting toxicity identified.
  - The magnitude of early T cell recovery (day +14) was associated with improved EFS.

Previous trials with lentiviral engineered T cells

- **HIV. Two trials completed.**
  - **Trial 1. 5 patients given a single infusion of autologous anti-sense env CD4 T cells. Doses: 0.6 to 1e10 cells/infusion. No SAEs**
  - **Trial 2. 18 patients given 3 or 6 infusions (total 56 infusions) of autologous anti-sense env CD4 T cells. Doses 0.5 to 1e10 cells/infusion. No SAEs**

Sustained Gene Transfer in Humans Using Lentiviral Vectors.

Levine et al. PNAS 2006

Analysis of Lentiviral Vector Integration in HIV+ Study Subjects Receiving Autologous Infusions of Gene Modified CD4+ T Cells

Wang et al, Mol Ther 2009

Rationale for omitting IL-2 infusions post T cell dosing

- CD3/28 stimulation promotes differentiation of T cells with Tcm phenotype that produce their own IL-2.
  - Levine et al. J Immunol 1997.
  - Bondanza et al. Blood 2006.
- IL-2 favors the outgrowth of Tregs (Malek et al. Nat Rev Immunol. 2004).
- Engraftment of CD3/28 stimulated CD4zeta CAR T cells in HIV patients is not augmented with IL-2 infusion (Mitsuyasu et al, Blood. 2000). Cells persisted for >5 years in 15 of 17 patients (Scholler et al, ISCT 2010).
- Preliminary data indicate robust engraftment of CD19 CARs in CLL patients with no IL-2 support:

Sample	% total leukocytes marked*
D-1	**
D0 post infusion	4.2
D+1	0.2
D+2	0.42
D+3	100
D+10	0.46
D+28	0.80
D+28 marrow	0.50
D+31 marrow	0.64

* Assumes single copy integration
** <0.1% (LOD)
50 ng DNA= DNA from c.a. 5,000 T cells

Expected safety profiles of engineered TCRs and CARs

- **CARs.**
  - **Potential for insertional oncogenesis.**
  - **Potential for autonomous proliferation and cytokine secretion.**
  - **SAEs reported.**

- **TCRs.**
  - **Potential for insertional oncogenesis.**
  - **TCRs use physiologic signaling and are subject to normal negative feedback through checkpoint inhibitors such as CTLA4 and PD1.**
  - **No SAEs have been reported with adoptively transferred CTLs expressing natural or engineered TCRs (>150 patients).**
  - **Cancer testis antigens have a favorable target product profile due to restricted expression than differentiation antigens like MART-1 and gp100.**

Previous and ongoing adoptive transfer studies with natural and genetically enhanced TCRs

Target Antigen	TCR Type	Indication	Study Chair, Center	ClinicalTrials.gov Identifier
CEA	anti-CEA TCR	metastatic cancer expressing CEA antigen	Steven A. Rosenberg, NCI - Surgery Branch	NCT00809978 NCT00923806
gp100	anti-gp100:154-162 TCR	melanoma	Steven A. Rosenberg, NCI - Surgery Branch	NCT00509496 NCT00610311
gp100 MART-1	Anti-gp 100:154 TCR Anti-MART-1 F5 TCR	melanoma	Steven A. Rosenberg, NCI - Surgery Branch	NCT00923195
MART-1 (DMF5)	TCR anti-MART-1 F5	High-risk melanoma	Steven A. Rosenberg, NCI - Surgery Branch	NCT00706992
MART-1	CTL line expressing TCR ant-MART-1	melanoma	Hans von der Maase, Aarhus University Hospital	
MART-1 (DMF4)	anti-MART-1 TCR-engineered TILs or PBLs	melanoma	Steven A. Rosenberg, NCI - Surgery Branch	NCT00091104

Previous and ongoing adoptive transfer studies with natural and genetically enhanced TCRs - II

Target Antigen	TCR Type	Indication	Study Chair, Center	ClinicalTrials.gov Identifier
MART-1 and gp100	anti-MART-1 and anti-gp100 TCR	melanoma	Steven A. Rosenberg, NCI - Surgery Branch	NCT00814684
NY ESO-1	anti-NY ESO-1 TCR	kidney cancer, melanoma, metastatic cancer expressing NY ESO-1	Steven A. Rosenberg, NCI - Surgery Branch	NCT00670748
NY-ESO-1	anti-NY ESO-1 TCR	Melanoma	Aude Chapuis-Fred Hutchinson Cancer Research Center	NCT00871481
p53	anti-p53 TCR	metastatic cancer with p53 overexpression	Steven A. Rosenberg, NCI - Surgery Branch	NCT00393029 NCT00704938
TRAIL Bound to the DR4 Receptor	Anti-TRAIL TCR	Metastatic Renal Cancer	James C. Yang NCI - Surgery Branch	NCT00923390

Previous Experience

Van Rhee F et al. NY-ESO-1 is highly expressed in poor-prognosis multiple myeloma and induces spontaneous humoral and cellular immune responses. Blood. 2005;105:3939.

Szmania S, et al. Immunization with a recombinant MAGE-A3 protein after high-dose therapy for myeloma. Journal of Immunotherapy. 2007;30:847.

Previous Experience - II

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).

Conclusions - I

- Cancer testis antigens have been demonstrated to have safety and potential benefit in the case of NY-ESO-1 TCR therapy. MAGE A3 has the same tissue expression pattern and is expected to have the same therapeutic index.
- Doses of 4x10e10 T cells in myeloma patients (n=50) have been shown to be feasible and well tolerated in myeloma patients after HSCT (Rapoport et al, 2009).
- NY-ESO-1^{c259} TCR transduced T cells have been given to melanoma patients after lymphodepleting chemotherapy and with high dose IL-2. The patients were dosed with a median of 5x10e10 T cells (range 1.6 - 13x10e10). There were no SAEs (Robbins, AACR 2010).

Conclusions - 2

- The above provides a sound rationale for a flat dose of 1e10 T cells transduced with NY-ES0-1^{c259} or MAGE A3^{a3a}.
- 1e10 T cells is likely the NOEL (no observed adverse effect level) dose, and the OBD (optimal biologic dose) is estimated to be ~5e10 T cells.
- Safety features of protocol 1056 include: 1) use of cancer testis antigens as a target; 2) previous demonstration that 4e10 T cells can be safely given to myeloma patients; 3) previous demonstration that 1.3x10e11 NY-ESO-1^{c259} T cells can be given to melanoma patients; 4) antigen is confined to the bone marrow and 5) omission of high dose IL-2 therapy.
- There is no precedent for dose escalation in trials with engineered TCRs

Questions?

Thank you for your time and effort